The Mechanism of Displacement of Sulphur from Adenosine 5'-[(*S*)α-thio-γ-benzyl] triphosphate

Gordon Lowe* and Graeme Semple

The Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, U.K.

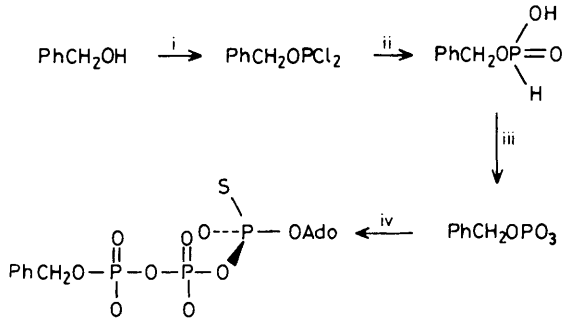
Adenosine 5'-[(*S*)α-thio-γ-benzyl]triphosphate on treatment with cyanogen bromide in [^{18}O]water gives [α-^{18}O]- and [γ-^{18}O]-adenosine 5'-[γ-benzyl]triphosphate in about equal amounts, the [α-^{18}O] material being formed with retention of configuration at phosphorus providing support for a mechanism involving participation by the γ-phosphate group.

During an attempt to prepare adenosine 5'-triphosphate (ATP) chirally labelled at P_α with ^{18}O, it was observed that when adenosine 5'-[(*S*)α-thio]triphosphate was treated with bromine in [^{18}O]water, ATP was produced in good yield but with the ^{18}O distributed between P_α and Pγ in the ratio of about 1 : 4. It was proposed that the [α-^{18}O]ATP was probably formed by direct replacement by [^{18}O]water on the bromine-activated adenosine 5'-[(*S*)α-thio]triphosphate, whereas the [γ-^{18}O]ATP was probably obtained by way of adenosine 5'-cyclotriphosphate.[1] This intermediate is known to ring open to give γ-substituted-ATP,[2] presumably because the phosphate diester is a better leaving group than the phosphate monoester. Similar studies with adenosine 5'-[(*S*)α-thio]diphosphate [(S_p)-ADP$_\alpha$*S*] have revealed that when the nucleotide is fully ionised and in buffered solution the β-phosphate residue participates intramolecularly with the cyanogen bromide activated thiophosphate to give adenosine 5'-cyclodiphosphate which ring opens to give ADP labelled at P_α and P_β.[3] By protecting P_β of the adenosine 5'-[(*S*)α-thio]diphosphate as its β-cyanoethyl ester, participation by the β-phosphate group was suppressed and the sulphur was displaced with inversion of configuration at phosphorus.[4]

In order to investigate whether protection of P_γ of adenosine 5'-[(*S*)α-thio]triphosphate would suppress participation of P_γ in the displacement of sulphur, adenosine 5'-[(*S*)α-thio,γ-benzyl]triphosphate was prepared from adenosine 5'-[(*S*)α-thio]diphosphate and benzyl phosphate as outlined in Scheme 1.

Adenosine 5'-[(*S*)α-thio,γ-benzyl]triphosphate on treatment with cyanogen bromide in [^{18}O]water gave adenosine 5'-[^{18}O,γ-benzyl]triphosphate (80% yield) which was shown by ^{31}P n.m.r. analysis to contain 50 atom % ^{18}O at P_α and 44 atom % ^{18}O at P_γ with no label at P_β. This implies that the γ-benzyl group is not suppressing participation by the γ-phosphate group, indeed both isotopomers could be derived *via* the intermediate benzyl adenosine 5'-cyclotriphosphate as outlined in Scheme 2. If so, the replacement of sulphur should proceed with overall retention of configuration at P_α.

Before investigating the stereochemistry at P_α of the adenosine 5'-[α-^{18}O,γ-benzyl]triphosphate derived from adenosine 5'-[(*S*)α-thio,γ-benzyl]triphosphate, (*N.B.*, the material is only 50% ^{18}O at P_α) a preliminary experiment was undertaken with unlabelled adenosine 5'-[γ-benzyl]triphosphate. Hydrolysis of this material in [^{18}O]water by snake venom phosphodiesterase gave [^{18}O]AMP as the only nucleotide product. Since the stereochemical course of hydrolysis by snake venom phosphodiesterase is known to proceed with retention of configuration,[8] adenosine 5'-[^{18}O, γ-benzyl]triphosphate was hydrolysed in [^{17}O]water with snake venom phosphodiesterase to give [^{16}O,^{17}O,^{18}O]AMP which was subjected to stereochemical analysis by ^{31}P n.m.r. spectroscopy after cyclisation and methylation.[9] If the [^{17}O]water and the P_α-^{18}O site had been fully enriched only one isotopomer of the esters would be observed in the ^{31}P n.m.r. spectrum owing to scalar relaxation caused by the ^{17}O when directly bonded to phosphorus.[10] In fact, the isotopic composition of the

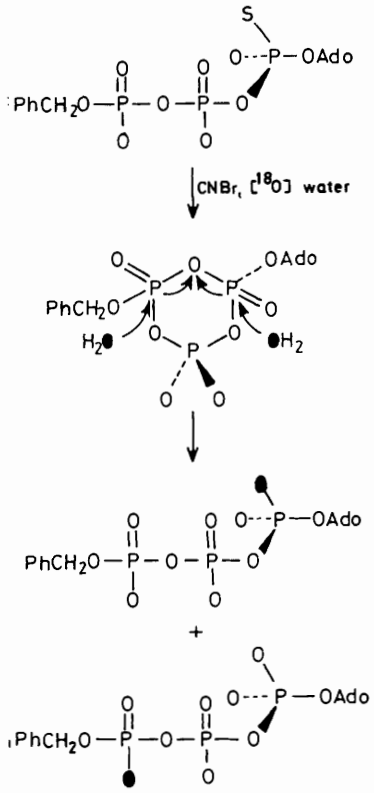


Scheme 1. Synthesis of adenosine 5'-[(*S*)α-thio,γ-benzyl]triphosphate. *Reagents*: i, PCl_3, MeCN (see ref. 5); ii, H_2O, Et_3N, tetrahydrofuran (THF); iii, (a) Me_3SiCl, Et_3N, (b) 2,2'-dipyridyl disulphide, (c) H_2O (see ref. 6); iv, (a) $(PhO)_2POCl$, (b) (S_p)-ADP$_\alpha$S (see ref. 7).

Table 1. Observed relative peak intensities of the ^{31}P n.m.r. resonances of the diastereoisomeric triesters derived by cyclisation and methylation of the 5'-[^{16}O,^{17}O,^{18}O]AMP, and the calculated values expected from the isotopic composition at P_α of the adenosine 5'[α-^{18}O,γ-benzyl]triphosphate (50 atom % ^{18}O) and the [^{17}O]water for retention and inversion of configuration at phosphorus.

	Equatorial triester			Axial triester		
	Observed	Calculated		Observed	Calculated	
Labelled triester		Retention	Inversion		Retention	Inversion
MeO–P=O	4.43[a]	2.50	2.50	2.54	2.50	2.50
Me●–P=O	0.71	0.73	1.00	1.00	1.00	0.73
MeO–P=●	1.00	1.00	0.73	0.75	0.73	1.00
Me●–P=●	0.19	0.10	0.10	0.20	0.10	0.10

[a] The line width and height of this peak suggested that it was enhanced by an impurity.



Scheme 2. The mechanism of displacement of sulphur from adenosine 5′-[(*S*)α-thio-γ-benzyl]triphosphate promoted by cyanogen bromide in [^{18}O]water.

[^{17}O]water was 61.7% ^{16}O, 28.0% ^{17}O, and 10.3% ^{18}O, and so all four isotopomers containing ^{16}O and ^{18}O are observed. The stereochemical evidence is nevertheless provided by the ratio of the two mono-^{18}O esters which is compared with the calculated ratios expected for retention and inversion of configuration in Table 1. Although it is not possible to exclude the possibility that some (<10%) of the displacement reaction has occurred directly with inversion of configuration, it is clear that the predominant if not exclusive mode of displacement is with *retention of configuration of phosphorus*. This provides strong support for the mechanism depicted in Scheme 2 in which the γ-phosphate group attacks the activated thiophosphate to give benzyl adenosine cyclotriphosphate which ring opens by attack of water with about equal probability at each of the phosphate triesters with the other phosphate triester acting as the leaving group.

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