E. coli Ada regulatory protein repairs the S_P diastereoisomer of alkylated DNA

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Using HPLC and ^{31}P NMR spectroscopy on a chemically synthesized asymmetric mixture of the diastereoisomers of thymidyl(3′→5′)thymidyl-*O*-methyl phosphate absolute configuration has been correlated with chromatographic mobility. The methyl phosphotriester system in alkylated DNA which is repaired by the Ada regulatory protein of *E. coli* has consequently been established to possess the S_p configuration.

Configurational analysis *HPLC* *^{31}P-NMR* *Phosphotriester repair*

1. INTRODUCTION

The isolation and purification of a 39 kDa *E. coli ada*$^+$ gene product which controls the adaptive response to alkylating agents has been reported recently [1]. As well as repairing O^6-methylguanine and O^4-methylthymine this protein has also been found to correct one of the stereoisomers of methyl phosphotriesters in alkylated DNA, such methylated centres existing in two diastereoisomeric forms, the simplest case being a dinucleoside phosphotriester (**1a/b**) (fig.1). This activity had been reported by McCarthy et al. [2] and was later shown to be a function of the Ada protein [3]. Which diastereoisomer is repaired, however, remains to be established, although in principle sufficient HPLC data have now been published [1] to allow solution of this problem by correlation with ^{31}P NMR assignments, which we report here. In view of the importance of establishing a correct configurational assignment to the understanding of the protein-nucleic acid interactions in this selective repair system we feel a rigorous stereochemical assignment to be essential.

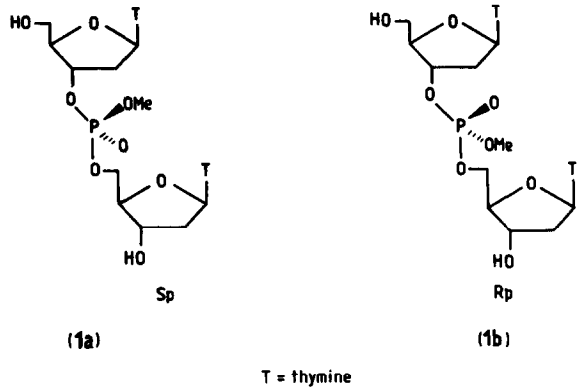

Fig.1. Diastereoisomeric forms of a dinucleoside phosphotriester.

2. MATERIALS AND METHODS

5′-*O*-Dimethoxytritylthymidyl(3′→5′)-3′-*O*-dimethoxytritylthymidyl-*O*-methyl phosphate was synthesized in a similar fashion to that of Uznanski et al. [4], except that the 5′-*O*-dimethoxytritylthymidyl-3′-*O*-diisopropylamino methoxy phosphoramidite was first prepared and coupled to 3′-dimethoxytritylthymidine according to Barone et al. [5]. The product after oxidation was chromatographed on a silica gel 60H flash column

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using chloroform as eluant. Partial separation of diastereoisomers was observed; our subsequent stereochemical assignments show that the S_p diastereoisomer has a slightly faster mobility on silica gel than the R_p. Detritylation of an appropriate pool of fractions using 80% acetic acid gave thymidyl(3′→5′)thymidyl-*O*-methyl phosphate (**1a/b**) as an unequal mixture of diastereoisomers as shown by ^{31}P NMR spectroscopy and HPLC.

^{31}P NMR spectra were recorded on a Jeol JNM-FX 60 spectrometer with broad band proton decoupling. Chemical shifts are relative to external 80% H_3PO_4; a positive shift indicates a downfield shift from this reference. HPLC was performed by isocratic elution on a Shimadzu LC4A liquid chromatograph using a Whatman Partisil PXS 10/25 C_{18} column with 20 mM triethylammonium bicarbonate, pH 7.5, and acetonitrile (7%) as the mobile phase.

3. RESULTS

Thymidyl(3′→5′)thymidyl-*O*-methyl phosphate (**1a/b**) was obtained from the chemical synthesis and chromatography as a mixture of diastereoisomers in unequal amounts. This was clear from the ^{31}P NMR spectrum (fig.2a) which indicates that the diastereoisomer resonating to lower field is present in a slight excess over that at higher field (~1.35:1). The configurations of these diastereoisomers have recently been assigned by ^{31}P NMR spectroscopy of specifically ^{18}O-labelled material in DMSO solution [6]. The diastereoisomer resonating to lower field was found to have the S_p configuration. Since this assignment is still valid when methanol is used as solvent (Potter, B.V.L., unpublished) the larger resonance in fig.2a is derived from the S_p diastereoisomer (**1a**).

The reverse-phase HPLC elution profile of the above diastereoisomeric mixture is shown in fig.2b. The ratio of the amounts of eluted diastereoisomers is ~1.26:1, 'fast':'slow', corresponding essentially to that observed in the ^{31}P NMR spectrum. The diastereoisomer present in excess is seen to elute first from the column and thus, in view of the above NMR correlation may be assigned the S_p configuration. Correspondingly, the slow diastereoisomer has the R_p configuration.

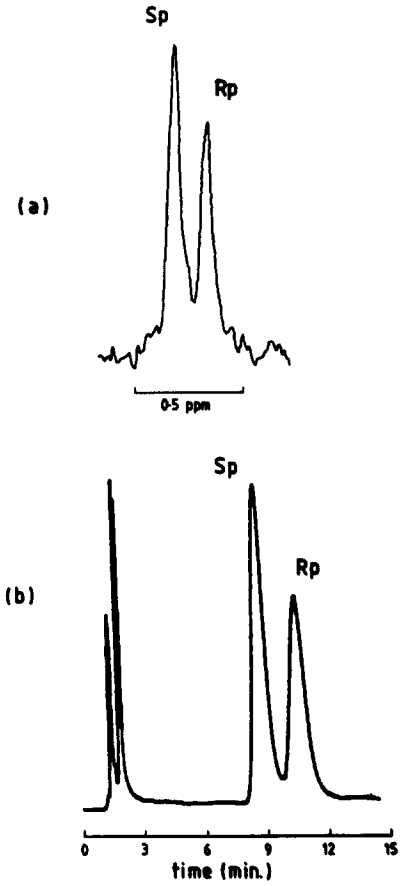

Fig.2. (a) 24.15 MHz ^{31}P NMR spectrum of the diastereoisomers of thymidyl(3′→5′)thymidyl-*O*-methyl phosphate recorded in methanol. ^{31}P NMR parameters were: sweep width, 500 Hz; pulse width, 4.6 μs; recorded in 4K; acquisition time, 4.09 s; no. of transients, 500. Chemical shifts of diastereoisomers, S_p, 2.00 ppm; R_p, 2.15 ppm. (b) Reverse-phase HPLC of the diastereoisomers (**1a/b**) performed as in section 2. Flow rate, 2 ml/min; retention times: S_p, 8.86 min; R_p, 10.76 min. (Unlabelled peaks are impurities eluting on the solvent front.)

4. DISCUSSION

Treatment of poly(dT)·poly(dA) with *N*-methyl-*N*-nitrosourea has been shown to generate methyl phosphotriesters in the dT strand as the predominant product (~90%) [1]. After treatment of this DNA with purified *Ada* protein 50% of the methyl phosphotriesters are repaired by transfer of the methyl group to a cysteine residue on the pro-

tein. Subsequent enzymic digestion of the DNA produces deoxyribonucleosides and the diastereoisomer of thymidyl(3′→5′)thymidyl-*O*-methyl phosphate resistant to repair. The two diastereoisomers of the latter (**1a/b**) can be separated by reverse-phase HPLC and designated fast and slow but no configurations can yet be correlated with these mobilities. It is the slow diastereoisomer which remains after DNA repair and digestion [1]. By chemical synthesis of an asymmetric mixture of (**1a/b**) we have been able to assign the HPLC peaks; the fast diastereoisomer possesses the S_p configuration and the slow diastereoisomer the R_p configuration. (Of interest is a recent report that the R_p diastereoisomer of thymidyl(3′→5′)thymidyl-*O*-isopropyl phosphate runs fast by reverse-phase HPLC [7]. In fact this diastereoisomer has the same relative configuration as S_p-(**1a**). A different group priority in applying the sequence rules of the *R,S* notation [8] (i.e. 5′-thymidyl > methyl whereas isopropyl > 5′-thymidyl) gives rise to the opposite *R,S* configurational assignment.) Thus we conclude that internucleotide phosphotriester linkages of the S_p configuration are repaired by the Ada protein. This result is in accord with a brief reference to an only tentative CD assignment [1], details of which have not yet been published.

Examination of a model of B-DNA shows that a methyl group accommodated on a *pro-S* internucleotide oxygen, which will protrude from the DNA, is likely to be more accessible to a DNA binding protein than one on the *pro-R* oxygen, where the methyl group will be located in the major groove of the DNA.

ACKNOWLEDGEMENT

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