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LC/ESI-MS/MS analysis of adducts formed in the reaction of 2-chloro-4-methylthiobutanoic acid with nucleosides

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2-Chloro-4-methylthiobutanoic acid (CMBA) is one of the most mutagenic substances extracted from salt-nitrite-treated Samna fish. The molecular mechanism pertaining to the mutagenicity of CMBA, however, remains unknown. We recently found that CMBA can react with 2'-deoxyguanosine (dG) in phosphate buffer (pH 7.4) to yield two CMBA-dG adducts (m/z 400/284) that may be associated with the mutagenicity.

In this study, we investigated the reaction of CMBA with other nucleosides (dA, T, dC) to determine any CMBA-nucleoside adducts. Experiments were carried out as follows. Each nucleoside (15 mM) was treated with CMBA (5 mM) in 200-OI of phosphate buffer (pH 7.4) at 37°C. At regular intervals during the 21-days incubation period, 25-μl aliquots were withdrawn from the reaction mixture and then stored at -80°C. Samples were thawed shortly before analysis, diluted 750-fold and 5-μl aliquots were injected into the liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI+-MS/MS). LC/ESI+-MS/MS analyses were performed by monitoring the precursor/product ion transitions of m/z 384/268 (for dA adducts), m/z 375/259 (for T adducts) and m/z 360/244 (for dC adducts). The results from the analysis showed that the reaction of CMBA with each nucleoside yielded respective adducts, two CMBA-dA adducts, one CMBA-T adduct and two CMBA-dC adducts.

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Analysis of DNA adducts formed with cholyl adenylate, a putative intermediate for biosynthesis of choly-CoA.

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Choloyl adenylate is known to be a putative intermediate for biosynthesis of cholic acid-coenzyme A (CoA) thioester conjugates by acyl-CoA synthetase. In the present study, the reaction of choly adenylate toward DNA under physiological conditions was analyzed. Choloyl adenylate was found to primarily attack hydroxy groups of ribose moieties of nucleosides. Moreover, exocyclic amino groups of 2'-deoxyctydine and 2'-deoxyadenosine were also found to serve as targets of choloyl adenylate, producing the corresponding cholic amides, N4-choloyl-2'-deoxyctydine and N6-choloyl-2'-deoxyadenosine. Structures of these DNA adducts were confirmed by direct comparison with synthetic compounds obtained from coupling reactions of cholic acid with each nucleoside in the presence of dicyclohexylcarbodiimide in pyridine at 70 °C. N4-Choloyl-2'-deoxyctydine was also obtained from enzymatic hydrolysates of calf thymus DNA reacted with choly adenylate. These results suggest that choly adenylate, if released from CoA synthetase, has the ability to modify DNA in vivo.

胆汁酸アデニレートより生成するDNA付加体の解析
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