P023 Structural analysis of DNA adducts formed from taurine with nitrite

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Taurine, 2-aminoethane-sulfonic acid, is known to exist at high concentrations in mammals, and conjugate with bile acids. In addition, taurine is present in various foods including squid, oyster and scallop. Recently, we demonstrated that *N*-nitrosotaurocholic acid reacts with 2'-deoxycytidine (dC) to form 3-ethanesulfonic acid-dC (3-ESA-dC) adduct. It is possible that taurine reacts with nitrite under acidic conditions to produce DNA adducts *in vivo*. Therefore, in the present study, we examined the formation and structure of nucleoside adducts in a reaction mixture of taurine, sodium nitrite ($NaNO_2$) and 2'-deoxynucleoside (dA, dC, dG, T) under acidic conditions. The adducts were analyzed by LC-ESI-MS. Several molecular ion peaks corresponding to the adducts were observed in the reaction mixtures containing dC or dG, however only traces were seen in dA or T reaction solutions. The major adduct detected in the dC mixture was identified to be 3-ESA-dC (yield, 0.4%) by comparison of its analytical data with the authentic compound. Moreover, the structure of one major adduct formed in the dG mixture was concluded to be *N*7-ESA-guanine (yield, 0.8%) by UV, mass and ^{1}H-NMR spectral analyses. The *in vivo* formation of these taurine-related DNA adducts from taurine and nitrite is now under investigation in our laboratory.

タウリンと亜硝酸の反応物から生成するDNA付加体の構造解析
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P024 Response to methylmethane sulfonate in Bhas 42 cell transformation at narrow concentration range

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Repeated trials for initiation assay of Bhas 42 cell transformation by MMS revealed that little or low number of transformed foci were induced depending on experiments. The object of the study was to clarify this uncertainty. Bhas 42 cells were cultured in DMEM/F12 added with 5% FBS (DF5F) or MEM added with 10% FBS (M10F). Cells were seeded into 6-well plates and treated with MMS for 3 days. The cultures were continued for 3 weeks in total and transformed foci were counted. For determination of cell growth crystal violet (CV) method and colony formation (CF) method were compared. CF method and CV method examined on Day 4 were compared with CV method examined on Day 7, the former two were more sensitive than the latter. Repeated experiments showed that use of M10F medium during MMS treatment induced transformed foci at a concentration range between 15 and 30 μg/mL and that of DF5F between 20 and 35 μg/mL. In some chemicals such as MMS, transformed foci were induced at a narrow concentration range. Two times dilution is too broad for examination of such chemicals. Does-spacing and top-dose selection are important factors to obtain reproducible results. CF method and CV method examined on Day 4 were too sensitive as the dose finding. Therefore, determination of cell growth on Day 7 by CV method is recommendable rather than CF assay.
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Bhas細胞を用いた形質転換試験における狭小毒性域でのメチルメタンスルフォネートの反応
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