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Thiophosphorylation of free amino acids and enzyme protein by thiophosphoramidate ions.

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Streszczenie

In search of an activity-preserving protein thiophosphorylation method, with thymidylate synthase recombinant protein used as a substrate, potassium thiophosphoramidate and diammonium thiophosphoramidate salts in Tris- and ammonium carbonate based buffer solutions were employed, proving to serve as a non-destructive environment. Using potassium phosphoramidate or diammonium thiophosphoramidate, a series of phosphorylated and thiophosphorylated amino acid derivatives was prepared, helping, together with computational (using density functional theory, DFT) estimation of ^{31}P NMR chemical shifts, to assign thiophosphorylated protein NMR resonances and prove the presence of thiophosphorylated lysine, serine and histidine moieties. Methods useful for prediction of ^{31}P NMR chemical shifts of thiophosphorylated amino acid moieties, and thiophosphates in general, are also presented. The preliminary results obtained from trypsin digestion of enzyme shows peak at *m/z* 1825.805 which is in perfect agreement with the simulated isotopic pattern distributions for monothiophosphate of TVQQQVHLNQDEYK where thiophosphate moiety is attached to histidine (His^{26}) or lysine (Lys^{33}) side-chain.

Słowa kluczowe

NMR, Thiophosphate, Tiophosphoramidate, Thiophosphorylation, Thymidylate synthase

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