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Nucleoside 5'-*O*-phosphorothioates as inhibitors for phosphatases

The close structural relationship between nucleoside 5'-*O*-phosphorothioates and nucleoside 5'-phosphates poses the interesting question of whether the former will show any interaction with phosphatases, either as substrates or inhibitors.

Recently we found 5'-TMPS ($pK_{a_2} = 4.80$) and 5'-UMPS ($pK_{a_2} = 4.60$) to be completely resistant to *Escherichia coli* alkaline phosphatase (EC 3.1.3.1), purchased from Sigma Chemical Co., U.S.A.. We now find 5'-TMPS also to be completely stable to acid phosphomonoesterase (EC 3.1.3.2) and acid phosphomonoesterase II, both from hog spleen, whose preparation and characterisation will be described by STERNBACH shortly[2].

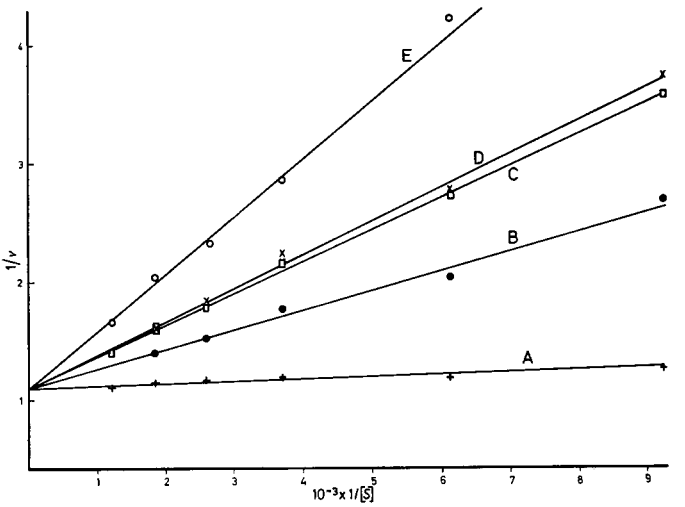


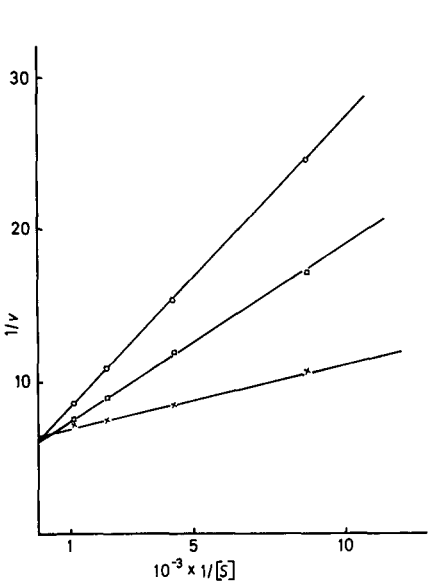
Fig. 1. Lineweaver–Burk plot of hydrolysis of *p*-nitrophenyl phosphate by *E. coli* alkaline phosphatase with 5'-TMPS and 5'-UMPS as inhibitors. Substrate and inhibitor concentrations in moles/l; velocity in μmoles/h per μg of protein. The reaction solution (3.0 ml) contained 0.3 mmole of Tris–HCl (pH 8.0), 0.5 μg of enzyme, *p*-nitrophenyl phosphate and inhibitor as indicated. The liberation of *p*-nitrophenol at 25° was followed over 12 min at 400 mμ with a Gilford recorder. A, no inhibitor; B, $1.15 \cdot 10^{-3}$ M 5'-UMPS; C, $1.45 \cdot 10^{-3}$ M 5'-TMPS; D, $2.15 \cdot 10^{-3}$ M 5'-UMPS; E, $2.75 \cdot 10^{-3}$ M 5'-TMPS. $K_m = 1.5 \cdot 10^{-5}$; K_i [UMPS] $= 1.2 \cdot 10^{-4}$; K_i [TMPS] $= 9.2 \cdot 10^{-5}$.

There is, however, an interaction between the phosphatases and the phosphorothioates as can be seen from Figs. 1–3.

Fig. 1 shows 5'-TMPS and 5'-UMPS to be competitive inhibitors of *E. coli* alkaline phosphatase contrary to our earlier erroneous finding[1]. 5'-TMPS is also a competitive inhibitor of the two acid phosphomonoesterases (Figs. 2 and 3).

These results reveal the strikingly different behaviours of S- and O-substituted esters of phosphorothioic acid towards *E. coli* alkaline phosphatase. Whereas the former are readily cleaved[3], the latter are competitive inhibitors. At this stage, all

Abbreviations: 5'-TMPS, thymidine 5'-*O*-phosphorothioate; 5'-UMPS, uridine 5'-*O*-phosphorothioate.



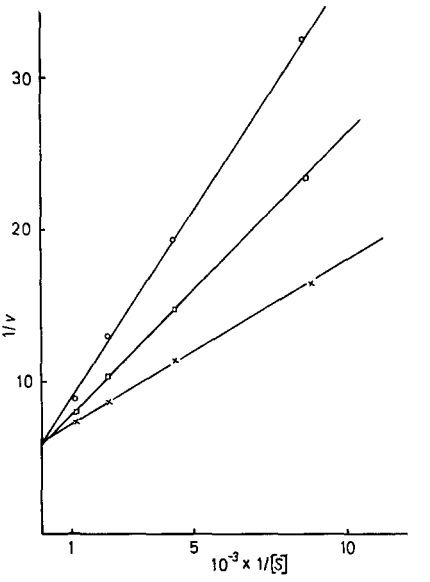


Fig. 2. Lineweaver–Burk plot of hydrolysis of *p*-nitrophenyl phosphate with acid phosphomonoesterase with 5′-TMPS as inhibitor. Substrate and inhibitor concentrations in moles/l; velocity in μmoles/ml per 10 min. The reaction solution (2.20 ml) contained 22 μmoles of EDTA, 330 μmoles of acetate buffer (pH 5.0), 0.020 ml of enzyme solution (7 μg of protein per ml), *p*-nitrophenyl phosphate and 5′-TMPS as indicated. The liberation of *p*-nitrophenol after 10 min at 37° was measured by adding 1.10 ml of reaction solution to 0.20 ml of 2 M NH_4OH and measuring the ultraviolet absorbance at 400 mμ. ×—×, no inhibitor; □—□, $2.67 \cdot 10^{-3}$ M 5′-TMPS; ○—○, $5.13 \cdot 10^{-3}$ M 5′-TMPS. $K_m = 7.2 \cdot 10^{-5}$; $K_i = 1.4 \cdot 10^{-3}$ respectively $3.0 \cdot 10^{-3}$.

Fig. 3. Lineweaver–Burk plot of hydrolysis of *p*-nitrophenyl phosphate with acid phosphomonoesterase II with 5′-TMPS as inhibitor. Conditions as for Fig. 2 except that the reaction solution contained only 0.010 ml of enzyme solution (11 μg of protein per ml). ×—×, no inhibitor; □—□, $0.75 \cdot 10^{-3}$ M 5′-TMPS; ○—○, $1.53 \cdot 10^{-3}$ M 5′-TMPS. $K_m = 1.9 \cdot 10^{-4}$; $K_i = 1.0 \cdot 10^{-3}$.

assumptions as to why O-substituted esters cannot be recognized as substrates are purely speculative. The higher polarizability, smaller electronegativity or larger size of sulfur, as well as possibly slightly different spatial requirements might contribute to this fact.

Nucleoside 5′-*O*-phosphorothioates should yield valuable information on the interaction of phosphatases with substrates.

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