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COMPARISON OF ACID- AND ENZYME-CATALYZED CLEAVAGE OF THE GLYCOSIDIC BOND OF N(7)-SUBSTITUTED GUANOSINES

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**Abstract:** Kinetic parameters for enzymatic cleavage of the glycosidic bond (phosphorolysis) of ten N(7)-substituted guanosines were determined, and used to establish a structure-activity relation for the Michaelis constants. Results were compared with those for acid-catalyzed cleavage of the glycosidic bonds, and are consistent with a mechanism for phosphorolysis via protonation of the purine ring N(7).

A study has been made of the effects of various substituents at N(7) of guanosine on susceptibility to acid-catalyzed and phosphorolytic (calf spleen purine nucleoside phosphorylase) cleavage of the glycosidic bond. The electronic properties of such substituents, expressed as Taft electronic constants  $\sigma^*$ ,<sup>1</sup> are correlated with the rate constants for acid-catalyzed depurination, which are increased by electron-withdrawing substituents.<sup>2</sup>

Kinetic parameters have now been determined for enzymatic phosphorolysis (followed spectrophotometrically) of ten N(7)-substituted guanosines,<sup>3,4</sup> methyl (m), ethyl (et), propyl (pr), isopropyl (ipr), butyl (bu), isobutyl (ibu), benzyl (bn), 1-phenylethyl (1phet), 2-phenylethyl (2phet) and carboxymethyl (cm). Correlation were then sought between the Michaelis constants  $K_m$  and the electronic, steric and hydrophobic properties of the substituents expressed in terms of the Taft electronic constants  $\sigma^*$ , the Taft steric constants  $E_s$ ,<sup>1</sup> and the Hansch constants,<sup>5</sup> respectively.

Since  $K_m$  for 7-methylguanosine is pH-dependent, the cation with a proton at N(1) being the preferred substrate, relative to the zwitterion,<sup>6</sup> correlations were based on  $K_m$  values for the cations,  $K_m^+$ , calculated as follows:

$$K_m^+ = K_m (10^{pH - pK_a} + 1)^{-1} \quad (1.1)$$

where  $K_m$  was determined at pH 7, and  $pK_a$  for dissociation of the proton at N(1) was calculated from the linear dependence of  $pK_a$  on  $\sigma^*$  of the N(7)-substituent.<sup>2</sup>

The "stepwise variable selection" procedure led to the following equation which best describes the observed values of  $K_m^+$ :

$$\log K_m^+ = 0.85(16.01) - 1.02(12.25) E_s + 9.55(5.24)(\sigma^*)^2 \quad (1.2)$$

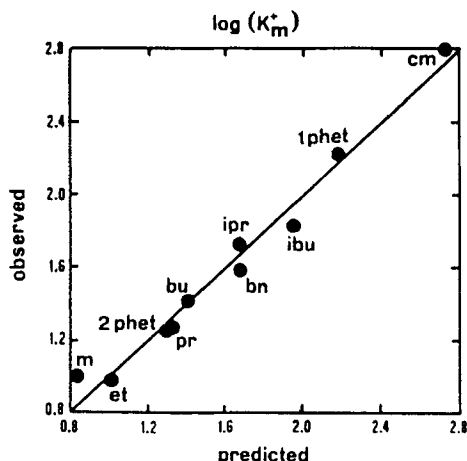


Fig. 1: Plot of experimental values of  $\log K_m^+$  for N(7)-substituted guanosines vs values predicted by equation (1.2).

with a correlation coefficient  $r = 0.989$ , adjusted for the number of degrees of freedom  $R = 0.986$ , with standard deviation  $s = 0.095$  and a Fischer coefficient  $F_{2,7} = 155.58$ . Figures in brackets are ratios of the fitted coefficients to their standard errors.

Note that there is no dependence of  $K_m^+$  on hydrophobic/hydrophilic properties; whereas an increase in steric hindrance, expressed in terms of  $E_s$ , leads to an increase in  $K_m^+$ . Appearance of the square of  $\sigma^*$  in the equation (1.2) points to protonation of the imidazole ring of guanosine, during phosphorolysis, since then both electron-withdrawing and electron-donating substituents result in an increase in the value of  $K_m^+$  (effect of deprotonation and protonation, respectively). The overall results suggest that, as for acid-catalyzed

hydrolysis of the glycosidic bond,<sup>7</sup> enzymatic phosphorolysis of guanosine proceeds via protonation of the purine ring N(7).

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#### REFERENCES

1. R.W.Taft (1956), "Steric Effects in Organic Chemistry", 556, M.S. Newman, Ed., Wiley, New York.
2. N. Muller and G. Eisenbrand (1985), *Chem.-Biol. Interactions* 53, 173.
3. A. Bzowska, E. Kulikowska, E. Darzynkiewicz and D. Shugar (1988), *J. Biol. Chem.* 263, 9212.
4. E. Darzynkiewicz, J. Stepinski, I. Ekiel, C. Goyer, N. Sonenberg, A. Temeriusz, Y. Jin, D. Haber and S.M. Tahara (1989), *Biochem.* (in press).
5. C. Hansch, A. Leo, S.H. Unger, K.H. Kim, D. Nikaitani and E.J. Lien (1973), *J. Med. Chem.* 16, 1207.
6. E. Kulikowska, A. Bzowska, J. Wierzchowski and D. Shugar (1986), *Biochim. Biophys Acta* 874, 355.
7. J.A. Zoltewicz, D.F. Clark, T.W. Sharpless and G. Grahe (1970), *J. Amer. Chem. Soc.* 92, 1741.