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QUANTITATIVE RELATIONSHIPS BETWEEN THE ELECTRONIC STRUCTURE AND BIOLOGICAL ACTIVITY OF SOME ANALOGUES OF OROTIC ACID

JOSÉ J. KANETI AND EVGENY V. GOLOVINSKY

Biochemical Research Laboratory, Bulgarian Academy of Sciences, 13 Sofia (Bulgaria)

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SUMMARY

The requirements for the chemical structure of the active antimetabolites of orotic acid (OA), established previously on the basis of the proposed model of the active site and mechanism of action of the enzyme orotidine-5′-monophosphate pyrophosphorylase, were used as the theoretical grounds for the synthesis of a number of analogues of OA. The activity of these analogue-antagonists has been investigated in the biosynthesis of pyrimidine nucleotides and in the growth of *Neurospora crassa*. It is shown that the biological activity of orotic acid analogues may be predicted semiquantitatively with the aid of quantum chemical data, *e.g.* frontier electron density f^e on the N-1 atom of the molecules, and by partition coefficients. A linear correlation was observed between the inhibition of [^{14}C]orotate incorporation into uridine monophosphate (UMP) and the $f^e{}_1$ of corresponding antimetabolites. The inhibition of *N. crassa* growth is also linearly correlated with the product of $f^e{}_1$ and a function of the partition coefficients.

INTRODUCTION

The reactions of OA and its analogues with PRPP have been considered from a theoretical point of view in a recent series of papers published by our group[1–6]. The well-known conditions of these reactions[7] have been used as a basis of a hypothetical model[1] of the active site of the enzyme oroditine-5′-phosphate pyrophosphorylase (EC 2.4.2.10). The conditions pertinent to the conversion of the analogues of OA to the corresponding nucleotides have been established on the basis of the

Abbreviations: MO, molecular orbital; OA, orotic acid; OMP, orotidine-5′-monophosphate; PRPP, 5-phosphoribosyl-1-pyrophosphate; UMP, uridine monophosphate.

same model[2]. Some of the analogues predicted to be active antimetabolites of OA have been synthesized in this laboratory and have been tested for biological activity. Two methods have been used in these experiments. The first one was an *in vivo* examination of the influence of the analogues on the growth of *Neurospora crassa*. This method has been used for the estimation of antimetabolic activity of the following compounds: 2,4-dihydroxypicolinic acid ('deazaorotic' acid)[3], 5-azaorotic acid[4], 2-hydroxy-4-amino-6-pyrimidinecarboxylic acid, 2-amino-4-hydroxy-6-pyrimidinecarboxylic acid, 2-mercapto-4-amino-6-pyrimidinecarboxylic acid, 2,4-dithioorotic acid and 2,4-diamino-6-pyrimidinecarboxylic acid[5]. 2-Thioorotic acid was tested by the same method.

The second method was an *in vitro* examination of the action of OA analogues on the biosynthesis of pyrimidine nucleotides. In these experiments we have estimated the inhibition of [^{14}C]orotate incorporation in UMP caused by the following compounds: 2-thioorotic acid, 5-azaorotic acid, 2,4-dihydroxypyrimidine-6-carboxylic acid[6], 2,4-dithioorotic acid and 2-hydroxy-4-amino-pyrimidine-6-carboxylic acid[5].

The purpose of the present study was to find some quantitative relationships between the observed antimetabolic activities of the above analogues of OA and their calculated electronic indices, such as atomic net charges, localisation energies, frontier electron densities, *etc.* In other words, we were looking for parallels between the antimetabolic activity and the chemical reactivity of the investigated compounds .We hoped to find some correlations resembling the linear free energy relationships.

Recently PULLMAN AND PULLMAN[8] pointed out that the active antimetabolites of the natural purine bases should have a net charge at the N-9 atom larger than a definite value. They have related this fact to the ability of the purine bases to react with PRPP to give the corresponding nucleotides. This relation is, however, of a qualitative character, since no enzymatic reactions have been considered. Our case was analogous but our purpose went further. That is why we were obliged to consider in detail the kinetic experiments concerning the incorporation of [^{14}C]orotate in UMP as well as to substantiate the choice of reactivity indices involved in these measurements.

METHODS

Theoretical basis

The kinetics of the reaction

$$OA + PRPP \rightleftarrows OMP + PP_i \xrightarrow{-CO_2} UMP$$

as mentioned above have been thoroughly studied[7]. Since the experiments included only one variable condition — the competitive inhibitor — it was reasonable to estimate the reaction rate from the yield of the product, UMP. The inhibition of the biosynthesis, *i.e.* the difference in the yields of labelled UMP in the experiments with an analogue and a control without it, may be taken as an estimate of the rate of a hypothetical conversion of the analogue into the corresponding nucleotide. In our model of the active site of OMP-pyrophosphorylase, we had suggested that the

binding ability of the substrate to the enzyme is determined by the electron affinity of the substrate. The electron affinities, *i.e.* in molecular orbital (MO) approximation the energies of the lowest empty MO's, of all compounds are nearly equal. Hence, it is reasonable to search for a correlation between the 'reactivity' of the analogues, *i.e.* their antimetabolic activity, and an electronic index of reactivity for these molecules.

Now we have to consider the reaction between OA and PRPP from the point of view of the quantum chemical theory of reactivity. It is known that when 2 molecules are brought together a mutual perturbation of the MO's occurs. This perturbation may be expressed[9,10] as:

$$\Delta E = \frac{2\,(c_r^m)^2\,(c_s^n)^2\,\beta^2}{E^{m*} - E^{n*}} \tag{1}$$

where c_r^m and c_s^n are the coefficients of the MO's with energies E^{m*} and E^{n*} on the atoms r and s, between which the new bond is forming; E^m is the energy of the highest occupied MO of the nucleophile (the donor); E^n is the energy of the lowest empty MO of the electrophilic agent (the acceptor); β represents the energy of the interaction between the orbitals. When the reaction takes place in a solvent, the total energy of the perturbation will be[9]:

$$\Delta E_{\text{total}} = -q_r q_s \frac{\Gamma}{\varepsilon} + \Delta_{\text{solv}} + \sum_{\substack{m \\ occ.}} \sum_{\substack{n \\ unocc.}} \frac{2\,(c_r^m)^2\,(c_s^n)^2\,\beta^2}{E^{m*} - E^{n*}} \tag{2}$$

The first term in (2) expresses the coulomb interaction of the charges q_r and q_s on the atoms r and s; Γ is a coulomb term taking into account the electronic repulsion; ε is the local dielectric constant of the solvent. The second term takes into account the changes in the energy of solvation. The third term expresses the charge transfer between the reacting molecules.

According to this treatment we can see that the energy of perturbation in our case will be defined essentially by the third term of (2), since q_r and q_s are small, $\varepsilon = 81$; Δ_{solv} may be considered constant for all analogues. The difference $E^{m*} - E^{n*}$, estimated by means of self-consistent MO calculations for OA[11] and extended Hückel calculations for PRPP[12], is also small; β is large since a covalent bond is formed as a result of the reaction. Consequently, the reaction considered is frontier controlled and we can endeavour to find a correlation between the frontier electron density[13] at the N-1 atom of the analogues and their antimetabolic activity. We note that the conclusion for the frontier control may be generalized for all similar biochemical reactions.

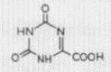
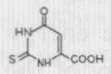
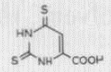
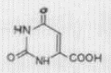
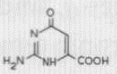
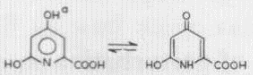
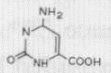
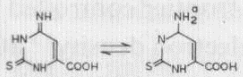
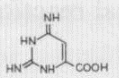
MO calculations

The MO calculations were performed by the simple Hückel approximation[14]. The parameter set used has been presented elsewhere[1,2,15] and has been proved to give satisfactory correlations with molecular data of the ground[16] and excited[15] states.

TABLE I

INDICES OF THE ELECTRONIC STRUCTURE OF SOME OA ANALOGUES

E_{res}, resonance energy; E^m, energy of the highest occupied MO; E^n, energy of the lowest empty MO; ln P, logarithm of the partition coefficient, $\ln P = 0.6670\,\Sigma S_r - 2.5395\,\Sigma Q_r + 0.4777$; $f^e{}_1$, frontier electron density at the N-1 atom in the highest occupied MO; $\pi = |\ln P_A - \ln P_{OA}|$; I_b, inhibition of UMP biosynthesis *in vitro*; $I_n{}^5$ and $I_n{}^{10}$, inhibition of the growth of *N. crassa* at concentration of analogues resp. 5 mM and 10 mM. The most stable tautomers are depicted (see text). Both tautomers are depicted if their E_{res} are close; the more stable form at low pH (experiments with *N. crassa*) is labelled by [a].

No.	*Compound*	$E_{res}\,\beta$	$E^m\,\beta$	$-E^n\,\beta$	ln P	$f^e{}_1$	$f^e{}_1 \cdot \pi$	I_b (%)	$I_n{}^5$ (%)	$I_n{}^{10}$ (%)
1		2.120	0.731	0.469	−2.349	0.604	0.0	—	—	—
2		3.339	0.582	0.469	−1.026	0.542	0.717	28	12	21
3		4.567	0.539	0.512	+0.535	0.600	1.730	20	17	54
4		2.951	0.913	0.400	−3.698	0.728	0.982	90	14	24
5		3.446	0.733	0.494	−0.486	0.580	1.081		8	43
6		4.853[a] 2.428	0.869 0.660	0.486 0.518	+1.079	0.041 0.435	0.142	0	0	0
7		3.454	0.728	0.496	−0.647	0.525	0.892	30	0	0
8		4.668[a] 4.630	0.527 0.538	0.497 0.496	+0.748 +0.413	0.399 0.581	1.236 1.605		4	13
9		5.460	0.575	0.458	−1.198	0.507	0.584		0	0

Since the compounds considered may exist in a number of tautomeric forms, calculations were made for every tautomer. The resonance energy was chosen as a criterion of the stability of the forms[8]. Tautomers with the largest E_{res} were considered preferable and are presented in Table I. In the case of oxygen functions, the lactam forms were considered. This is based on the fact that in the Hückel approximation the carbonyl group possesses an additional energy of approx. 14 kcal/mole per C=O group[8]. This energy calculated by means of our parameter set is on the oder of 1.3 β, estimated from the equilibrium 9-anthrol $\rightleftharpoons$ anthrone[8,17]. Thus, all compounds have nearly equal resonance energies in the range 4.5–5.5 β (see Table I).

In the case of 2,4-dihydroxypicolinic ('3-deazaorotic') acid, the most stable aromatic form and one of the lactam forms were taken into consideration. This compound has recently been predicted[2] to be inactive as an antimetabolite of OA. It was chosen to estimate the lower threshold value of the reactivity index necessary for displaying antimetabolic activity. There are some indications that the equilibrium between the aromatic and lactam forms of the latter compound is measurable under physiological conditions (D-exchange in the corresponding nucleotide[18]). That is why we have chosen the most reactive lactam form to be the zero point of our correlation between the reactivity indices and the inhibition of pyrimidine biosynthesis.

For the correlation between electronic structure and the influence of the compounds on the growth of *N. crassa*, we have calculated their partition coefficients between water and 1-octanol[19]. This system has been recommended to be the best model of permeation through the cell walls[20]. The partition coefficients measured for this system have been shown to give satisfactory correlations for drug activities in combination with the Hammett equation[20,21].

RESULTS AND DISCUSSION

Fig. 1 depicts a plot of the inhibition of the biosynthesis of UMP against the frontier electron densities $f^e{}_1$ at the N-1 atom of the corresponding compounds. We can see that the antimetabolic activity is linearly dependent on the reactivity indices of the molecules. This is indirect evidence for the correctness of our assumption that the active antimetabolites of OA are those able to form nucleotides with PRPP. At present this statement has been directly confirmed only for 5-azaorotic acid[22] and 5-fluoroorotic acid[23].

The correlation between the antimetabolic activity and electronic structure may be strengthened by taking into consideration the different binding ability of the substrates to the enzyme. We can tentatively correlate the inhibition of UMP biosynthesis with the product:

$$f^e{}_1 \{1 - \text{const.}\,(E^n{}_{OA} - E^n{}_A)\}$$

symbols as in (1) and (2); OA, orotic acid; A, analogue.

It is possible in this manner to take into account the binding between the enzyme and the substrate according to our model of the active site[1]. If we make const. = 1, we can see that even this rough approximation obviously improves our

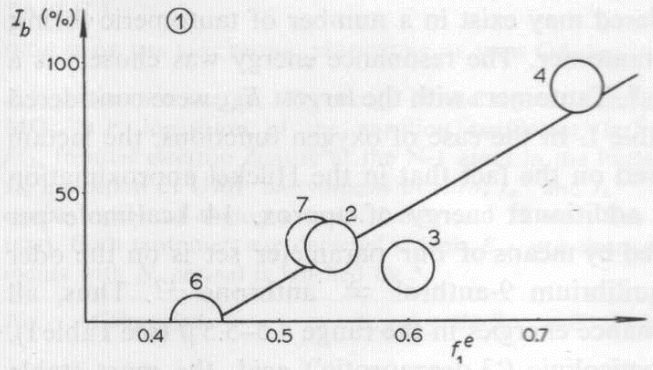


Fig. 1. Inhibition of UMP biosynthesis *vs.* frontier electron density $f^e{}_1$ of the analogues. Concentration of OA analogues 2.5 mM; concentration of [^{14}C]orotate 1.5–2 mM; pH = 7.6. $I_b = (2.91\, f^e{}_1 - 1.312) \cdot 100\%$, correlation coefficient $r = 0.89$; $I_b = 0$ when $f^e{}_1 = 0.451$ (the lower threshold value). For the numbering of analogues see Table I.

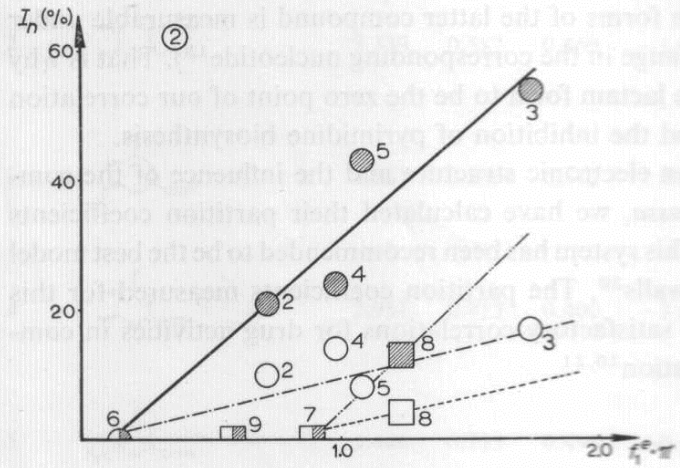


Fig. 2. Inhibition of the growth of *Neurospora crassa* by OA analogues *vs.* the function $f^e{}_1 \cdot \pi$. Circles, compounds substituted at the 4th position with O or S; squares, compounds substituted at the 4th position with NH. Black figures, concentration of the analogues 10 mM; white figures, concentration of the analogues 5 mM; pH = 5.6. Correlations established for oxo and thio compounds are: $I_n{}^5 = (0.097\, f^e{}_1 \cdot \pi - 0.003) \cdot 100\%$, correlation coefficient $r = 0.87$; $I_n{}^{10} = (0.35\, f^e{}_1 \cdot \pi - 0.041) \cdot 100\%$, correlation coefficient $r = 0.97$; $I_n{}^{10} = 0$ when $f^e{}_1 \cdot \pi = 0.117$ (the lower threshold value). For the numbering of analogues see Table I.

correlation — the deviations of 2,4-dithioorotic acid and 5-azaorotic acid from the correlation straight line become smaller (see Table I).

We can now consider the experiments concerning the growth of *N. crassa* in the presence of the same antimetabolites. It is known that the rate of permeation through the cell wall is a logarithmic function of the partition coefficients of the compounds between water and any organic phase[20,21]. Evidently the permeation rate determines the probability of a molecule to reach the enzyme and, further, to bind with it and to be metabolized. Hence, we could expect a correlation between the inhibition of the growth of *N. crassa* and the product:

$$f^e{}_1 \cdot \ln P_A \quad \text{or} \quad f^e{}_1 (\ln P_A - \ln P_{OA})$$

where P_A and P_{OA} are the corresponding partition coefficients. In fact, we have found that the influence of OA antimetabolites on the development of Neurospora may be expressed through the product

$$f^e_1 \cdot \pi = f^e_1 \, |\ln P_A - \ln P_{OA}|$$

This relation is plotted in Fig. 2 for two concentrations of the inhibitors.

We can see from Fig. 2 that the antimetabolites examined on *N. crassa* may be divided into two classes: *(1)* those substituted at the 4th position with O or S, and *(2)* these substituted in the 4th position with NH. The oxo and thio compounds, more closely resembling the normal metabolite, are significantly more active than the amino compounds. Since such a distinction has not been observed for the same compounds in their influence on UMP biosynthesis, this feature may be regarded as a consequence of 2 alternatives. The first alternative involves the well-known shortcomings of the Hückel MO's in the calculation of atomic π-charges[24] and consequently also in the calculations of partition coefficients. The second alternative is that this division may be an effect of actually existing peculiarities in the transport of these compounds through the cell walls. At present it is impossible to interpret this fact unambiguously.

It is relevant to consider also the differences between the functions $\pi = \ln P_A - \ln P_{OA}$, recommended in the literature[20], and $\pi = |\ln P_A - \ln P_{OA}|$, found in our experiments. As may be seen in Table I, the only compound with $\ln P_A$ smaller than that of OA is 5-azaorotic acid. The successful attempt to correlate the antimetabolic effect of the compounds with $\pi = |\ln P_A - \ln P_{OA}|$ may be fortuitous for azaorotic acid or may again be a consequence of peculiarities in the transport through the cell walls. Again, it is impossible to interpret this fact unambiguously.

CONCLUSIONS

On the basis of the quantum chemical theory of reactivity, it is shown that the reaction between OA and PRPP should be frontier controlled. On these grounds, a correlation between frontier electron density f^e at the N-1 atom of some OA analogues and the biological activity of the latter was established.

The relationships between the electronic structure and antimetabolic activity permit the semiquantitative prediction of the behaviour of any analogue of OA in UMP biosynthesis *in vitro* as well as in the experiments on microorganisms *in vivo*. The active antimetabolites of OA must meet the requirements for the chemical structure established previously[2] as well as the requirements for the chemical reactivity, defined by the electronic structure. The lower threshold value of the reactivity index f^e_1 for the active compounds is 0.45. The lower threshold value of the product $f^e_1 \cdot \pi$ of the compounds inhibiting the growth of *Neurospora crassa* is 0.12.

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