Molecular Orbital Calculations on Folic Acid and Folic Acid Analogs

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(Received September 14, 1966)

SUMMARY

In an attempt to understand the mechanism by which antifolate agents bind to dihydrofolate reductase, it became necessary to examine existing inhibitors in greater detail. This paper is a result of an investigation of such inhibitors by means of molecular orbital calculations. The main point of departure from previous studies (9, 10) is that the enol form of the 2-amino, 4-hydroxy, pteridines has been assumed to be the active isomer. With this assumption the anomalous action of 2-amino, 4-hydroxy, 6-formyl pteridine has been explained. The present investigation gives further support to the basicity hypothesis as formulated by Pullman *et al.* (10).

INTRODUCTION

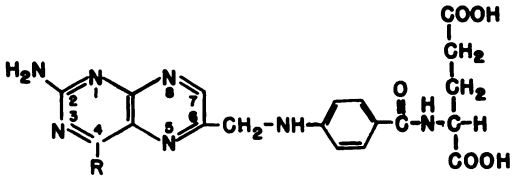
The reactions that will be discussed in this paper occur in the DHFA[1] reductase system. The importance of this enzyme and the many reactions involving FA (Fig. 1, Compound I) have been well documented (for a review see references 1, 2). Our interest in this key enzyme was in the structure-activity relationships among some of the inhibitors. The tenacious binding of aminopterin (Fig. 1, Compound II) to the enzyme, with the resultant inhibition, is probably the single most important action of this drug (1). It is this remarkable affinity of aminopterin for DHFA reductase that caused us to focus our attention on the pteridine analogs in order to determine what features are necessary for optimum binding.

METHODS

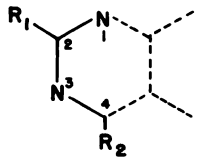
The method employed for the calculations of the π-electronic charge distribution were carried out by the Hückel molecular orbital method of quantum chemistry. This technique has been described in the book by Pullman and Pullman (3). The parameters used for the coulomb and bond integrals are given in our previous publication (4). The basicities of the ring nitrogens were calculated by the method of Nakajima and Pullman (5) and are reported as

$$-\Sigma Q_p(dd/pp) \qquad (1)$$

where Q_p is the net π charge on atom p and (dd/pp) is the coulomb integral between an electron of the lone pair orbital d on the nitrogen and the electron of the π orbital



I; R=OH
II; R=NH2



III

FIG. 1. *Formulas of key molecules discussed in this report*

[1] The abbreviations used in this report are: DHFA, 7,8-dehydrofolic acid; FA, folic acid; THFA, tetrahydrofolic acid; LCAO, linear combination of atomic orbitals.

on atom p. The summation is taken over all atoms p of the π system. The calculation of (dd/pp) is based on the set of equations described by Pariser and Parr (6) for the approximate evaluation of this integral. The relation shown in formula 1 is related to the pK of the nitrogen through equation 2

$$pK = B - C\Sigma Q_p(dd/pp) \qquad (2)$$

where B and C are constants for a series of molecules in the same family (5). For comparing basicities, the expression in formula (1) may be used directly to estimate the relative values for the different nitrogens.

The calculations in this paper have all been performed on the pteridine moiety. The contribution of the *p*-aminobenzoyl-L-glutamate to the electronic densities has been ignored; the reason for this will be discussed below.

THE MODEL

A great deal has been written on the specific sites which are necessary for binding of FA or any of the inhibitors such as aminopterin (Fig. 1, Compound II) to DHFA reductase. Only a few references will be cited: those by Baker (7, 8) Pullman (9, 10), and Zakrzewski (11) and their respective co-workers. The purpose of this paper is to present a model that will explain some of the anomalies that have been noted in the past.

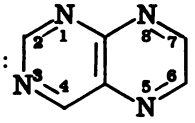
FA may manifest keto-enol tautomerism, and it is important to decide which isomer is bound to the enzyme surface. Since aminopterin is bound more tightly than folic acid, I would like to suggest that the 4-amino is important for binding, in addition to the 2-amino and N_1 of the pteridine ring (9, 12). Molecular orbital calculations on aminopterin have been described by Pullman (9), and the pertinent values for the pteridine residue are shown in Table 1. These results show that the 4-amino group has a net positive charge. The keto form of FA, while it is undoubtedly the most stable, has an excess of electrons on the 4 substituent (Table 1, Compound 2) which would cause repulsion rather than attraction. The enol form, on the other hand (Table 1, Compound 1), has a net positive charge and should be attracted to the enzyme surface. It appears feasible for the enzyme to bind one isomer and thus drive the keto-enol equilibrium in the direction of more enol, which is preferred by the catalytic site. This hypothesis regarding the enol form receives support from the data of Zakrzewski (11). He demonstrated that the folate enzyme complex has a ΔS of 13 cal/degree for the dissociation reaction. On the other hand, the various inhibitors, including aminopterin and 2,4-diaminopteridine, have a ΔS of -10 cal/degree. From an analysis of these data, Zakrzewski (11) concluded that the positive entropy change for the dissociation of reductase–folate complex was related to the rearrangement of the substrate rather than the enzyme. This might be explained by the folate spontaneously assuming its more stable keto form upon dissociation with the resulting entropy increase. Zakrzewski's conclusion regarding the nonparticipation of the *p*-aminobenzoyl-L-glutamate moiety to the entropy change for folic acid (11) is partially supported by the work of Baker *et al.* (7). These authors studied the binding of various analogs to DHFA reductase and reached a conclusion regarding the nonparticipation of the carboxy-L-glutamate to the binding of the inhibitor to the enzyme. Baker *et al.* (8) made the further suggestion that the importance of the glutamate portion of aminopterin might be connected with active transport of the drug to the site of action.

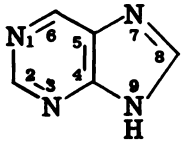
With these considerations in mind, I would like to extend the critical binding sites proposed by Pullman (3, 10) to include the 4-substituent as illustrated in Fig. 1, Compound III. These factors may be summarized as follows: (a) N_1 must be basic enough to have affinity for a proton-donating center on the enzyme. (b) R_1 and R_2 must have a net positive charge in order to be attracted to an electron-rich site on on the enzyme.

A number of analogs will be reexamined using this picture for binding to DHFA reductase. In this study, the calculations on the 4-hydroxy derivatives will be based on

TABLE 1

Electronic parameters for various folic acid analogs calculated by means of LCAO

Compounds 1–12: 

Compounds 13–15: 

Compound	Basicity $-\Sigma Q_p$ (pp/dd) N_1	Electron density 2-NH_2	4-NH_2	4-OH	C_6	C_7	DHFA reductase inhibition[a] K_i
1 2-amino, 4-hydroxy (enol)	2.15	0.188	—	0.102	0.042	0.099	—
2 2-amino, 4-hydroxy (keto)	1.23	0.209	—	−0.426	0.010	0.085	—
3 2-amino, 4-hydroxy, 7,8-dihydro	1.91	0.185	—	0.092	0.049	—	—
4 2-amino, 4-hydroxy, 5,6,7,8-tetrahydro	2.21	0.163	—	0.079	—	—	—
5 2,4-diamino	2.48	0.187	0.219	—	0.033	0.095	4.7×10^{-7}
6 2,4-diamino, 7,8-dihydro	1.94	0.183	0.185	—	0.156	—	—
7 2,4-diamino, 5,6,7,8-tetrahydro	2.51	0.163	0.160	—	—	—	—
8 2,4-diamino, 6-formyl	2.16	0.191	0.223	—	0.002	0.120	8.1×10^{-6}
9 2,4-diamino, 6-formyl, 7,8-dihydro	1.67	0.192	0.201	—	−.043	—	—
10 2,amino, 4-hydroxy, 6-formyl	2.06	0.193	—	0.104	0.010	0.123	2.9×10^{-4}
11 2,amino, 4-hydroxy, 6-formyl, 7,8-dihydro	1.23	0.192	—	0.096	−.027	—	—
12 2,4-diamino, 6-hydroxy	2.01	0.168	0.198	—	0.200	0.074	9.0×10^{-5}
13 2,6-diamino	2.22[b]						1.5×10^{-6}
14 2-amino	1.39[b]						Inert
15 2-amino, 6-hydroxy	1.70						Inert

[a] Data taken from Ref. 11.

[b] Data taken from Ref. 10.

the premise that the active form of the molecule is in the enol form.

RESULTS AND DISCUSSION

The substrate is bound to the enzyme through N_1, 2-amino and the 4-hydroxy group. As FA becomes reduced to THFA, the positive charge on the 4-hydroxy is reduced to the point where the molecule no longer is bound. Although the basicity of N_1 is still high, it is not enough to hold the molecule to the protein surface, and room is made for the attachment of fresh substrate. In addition to the loosening of the molecule by the reduction in charge, there will be a negative ΔF associated with the conversion of the enol to the more stable keto isomer. This free energy change, which is reflected in the positive ΔS given by Zakrzewski (11) for the dissociation of folate-reductase system, will contribute a further driving force in removing the reduced substrate from the enzyme. The various inhibitors, especially the 2,4-diamino-substituted molecules, have a greater affinity for the enzyme due to a greater basicity of N_1 and a greater positive charge on the 4-amino group. Even if these molecules become reduced, they still have sufficient binding strength to remain associated with the enzyme. This is illustrated by the fact that the charge on the 4-amino in fully reduced 2,4-diamino pteridine (compound 7, Table 1) is greater than the charge on the oxidized form of the substrate (Compound 1, Table 1). In addition, the inhibitors do not have the free energy factor to drive them off the enzyme. The 2-amino, 4-hydroxy, 6-formyl pteridine (Compound 10, Table 1) is an interesting inhibitor.

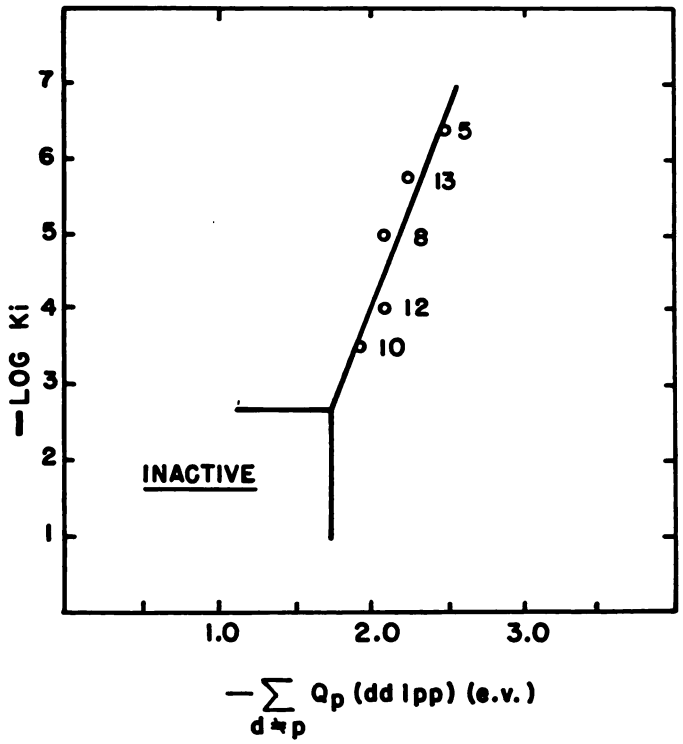


FIG. 2. *The correlation between basicity and —log K_i where K_i is the inhibition constant reported in Table 2*

The numbers refer to the compounds listed in Table 2.

Neither Zakrzewski (11) nor Collin and Pullman (10) were able to account for this observation in a satisfactory matter. By considering the molecule in the enol form, I would like to suggest that this material is absorbed to the enzyme in a manner similar to folic acid. The enzymic transfer of hydrogen from NADPH to the formyl pteridine is accompanied by the disappearance of the aldehyde function (13). Consequently, the product that is formed is not the 7,8-dihydro analog. The analytical data (13) indicate that the product of this reaction results from the transfer of only two hydrogens. This reduced material must have an affinity for the active site on the DHFA reductase in order for inhibition to result.

From these data, it would appear that an inhibitor must maintain a positive charge on 4-substituent of approximately 0.1 and a basicity on the N_1 of —1.7 or greater. If either value falls below these limits, the molecule will lose its affinity for the protein and become dissociated. Not too much can be said concerning the 2-amino group since the examples cited do not show much variation in charge on this group. Figure 2 graphically displays the inhibition data of Zakrzewski (11) plotted against the basicity of N_1. As may be noticed, there is a good correlation which lends further support to the basicity hypothesis as advanced by Pullman *et al.* (9, 10).

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