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Modulation of interleukin 2 high affinity binding to human T cells by a pyrimidodiazepine insect metabolite

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An insect metabolite containing the little known pyrimido[4,5-b][1,4]diazepine ring system has been found to act as an effective mimic of tetrahydrobiopterin in its ability to modulate the affinity of interleukin 2 (IL-2) for its receptors on human T cells. Semi-empirical molecular orbital calculations reveal that while tetrahydrobiopterin has considerable flexibility, the pyrimidodiazepine has rather few conformational options and offers a useful model for exploring the nature of the pterin binding site.

Interleukin 2 receptor; Tetrahydrobiopterin; Homopterin; Pyrimido[4,5-b][1,4]diazepine; Molecular modelling; Conformational analysis

1. INTRODUCTION

Tetrahydrobiopterin (Scheme 1 (1)) is well established as the essential co-factor for the hydroxylation of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan [1], thus playing an essential role in neurotransmitter biosynthesis and phenylalanine degradation. It also serves as a cofactor for the cleavage of glyceryl ethers [2], and is involved in the formation of nitric oxide from L-arginine [3]. Recently, however, it was discovered that it is synthesised in cells which lack any of these known biological functions, e.g. in hematopoietic progenitor cells [4,5], and activated T cells [6], and a new function for it emerged when it was found that it can act as a growth regulator for both of these cell types [4,7–9]. In T cells, the mechanism for this regulatory process of tetrahydrobiopterin (1) involves an enhanced affinity of the receptor for IL-2. This effect was first noticed [10] in murine cyotoxic T cells, and was then observed [11] in the human T cell line MT-2. In the latter case the response was found to have considerable structural specificity, and in a study employing ten pteridines, (6*R*)-5,6,7,8-tetrahydrobiopterin (1) was alone in causing a significant increase in receptor affinity for IL-2. Of the other compounds tested, only biopterin had any measurable effect, and that was slight. In this communication we report that an insect metabolite (Scheme 1 (2)) containing the little known pyrimido[4,5-b][1,4]diazepine ring system can act as an effective mimic of tetrahydrobiopterin (1) in enhancing the affinity of the IL-2 receptor in human T cells. Furthermore,

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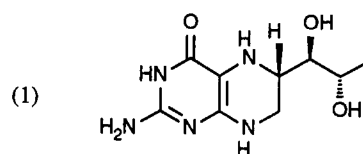

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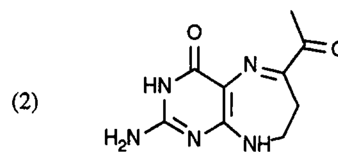

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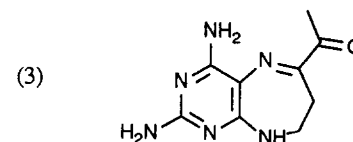

Scheme 1.

when stable conformers of the two molecules were identified and compared using semi empirical molecular orbital calculations, close spatial correspondences between the two were revealed, allowing possible inferences as to the nature of the binding site.

2. MATERIALS AND METHODS

(6*R*)-5,6,7,8-Tetrahydrobiopterin (1) was obtained from Dr. B. Schircks, Jona Laboratories, Switzerland. 6-Acetyl-2-amino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepin-4(3*H*)-one (2), and 6-acetyl-2,4-diamino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepine (3) were synthesised as previously described by Boyle et al. [12–14].

The K_d values shown in Fig. 1 were obtained by Scatchard analysis of equilibrium binding of [^{125}I]interleukin 2 to the MT-2 cells. The origin and methods of long term cultivation of the CD4$^+$ T cell line MT-2 are described in reference [6]. For equilibrium binding of the ligand, cells were suspended in RPMI 1640 plus 1% bovine serum albumin, transferred into Remova strip U-form wells (3×10^5 cells per well) and incubated at 4°C for 40 min with serial dilutions of [^{125}I]interleukin 2 as described in reference [11]. Tetrahydrobiopterin (1), the pyrimidodiazepine (2), and the 4-aminopyrimidodiazepine (3) (Scheme 1) were added at a concentration of 3×10^{-7} M. Scatchard analysis of the equilibrium binding data (triplicate determinations, 5–7 independent experiments) was performed with the LIGAND computer program system (1983 revision) together with the operation guide (1987 version) kindly provided by Dr. P.I. Munson, (National Institute of Child Health and Human Development, Bethesda, MD 20205). The program allows pooling of multiple experiments and provides calculation of confidence intervals. Details of its principles are described in reference [15] and outlined in reference [10].

Energy calculations were performed using the PM3 semi-empirical molecular orbital method [16] as implemented in the MOPAC computer programme; running on a Silicon Graphics IRIS 4D25TG workstation. Energy minima were located using maximally stringent convergence criteria ('precise' mode). The initial structures were assembled using the QUANTA molecular modelling package, supplied by Molecular Simulations Inc., Waltham, MA. This software was also used for visualisation and comparison of structures. The structure matching procedure which yielded the configurations as shown in Fig. 2 was performed as follows. Energy minimisation of pyrimidodiazepine (2) yielded conformers in which the O=C-C-CH_2 dihedral angle, θ, took values of −62.4° and +59.7° (ΔH_f = −38.664 and −38.329 kcal·mol^{-1}, respectively). However, the minima were found to be shallow with respect to variation of θ, distortions of ± 20° requiring ≤ 0.3 kcal·mol^{-1}. In attempting to match these conformations of (2) to tetrahydrobiopterin (1) it was immediately apparent that the side- chain of the latter must be disposed pseudo-equatorially. Candidate structures for tetrahydrobiopterin (1) were generated by systematic rotations about the side chain C-C bonds, visual comparison with (2) (bearing in mind the range of angles available to θ), and energy minimisation in promising cases. In only one case was a close correspondence found *both* between the overall dispositions of the side- chains, *and* between the positions of the carbonyl oxygen in (2) and one of the hydroxyl oxygens in (1). This conformational minimum for tetrahydrobiopterin (1) was selected and is shown in Fig. 2a. The corresponding conformation for pyrimidodiazepine (2), shown in Fig. 2b, was generated by least squares matching of the asterisked atoms, allowing variation of θ. The RMS difference for the positions of the superposed atoms was < 0.2 Å.

3. RESULTS AND DISCUSSION

An integral part of the cellular immune response mechanism is the clonal expansion of T cells induced by the binding of interleukin 2 (IL-2) to specific high affinity receptors [17–19]. It was discovered recently that (6*R*)-tetrahydrobiopterin, which is produced by the T cells [6], modulates the affinity of these receptors for IL-2 [10], and this constitutes a feedback mechanism resulting in an enhanced entry of the T cells into the DNA synthesis phase. As already mentioned, several pteridines closely related to tetrahydrobiopterin failed to give a similar effect [11]. The present experiments show, however, that the pyrimidodiazepine (2), which occurs naturally as an insect metabolite, is almost as effective as tetrahydrobiopterin (1) in its ability to promote the affinity of the receptors for IL-2.

The insect metabolite (2) used in this work was first isolated [20] in 1977 from the heads of *Drosophila melanogaster*. It is biosynthetically related to tetrahydrobiopterin (1) in that the two molecules share a common precursor [21]. However, it is structurally rather different [22,23] and is, in effect, a 'homopteridine'. Heretofore its only known biological significance [21,24] has been as an intermediate on the biogenetic pathway to the drosopterins, the red eye pigments of the insect. Until recently any further investigations of its properties were hampered by lack of material, but its chemical synthesis [12] has made available sufficient quantities for the present experiments. Its 4-amino analogue (3), was also synthesised [13,14] and tested, since it is well known that some of the most effective folic acid antagonists are those in which the pterin 4-oxo group has been replaced by a 4-amino group. In our experiments, we used the HTLV-I infected CD4$^+$ T cell line MT-2, firstly because it expresses both chains of the IL-2 high affinity receptor [18,25] and secondly, because it lacks constitutive tetrahydrobiopterin synthesis [6]. The apparent affinity of the IL-2 receptor was measured by determining the K_d values of [^{125}I]IL-2, and the results are shown in Fig. 1. As can be seen, the K_d value of the control MT-2 cells decreased by about half when either tetrahydrobiopterin (1) or the insect metabolite (2) was added. In other words, the affinity of the receptor for IL-2 was about doubled in presence of either of these compounds. The amino analogue (3) had no effect.

The cellular immune response depends upon proper control of T cell amplification. It is initiated by IL-2 binding to its high affinity receptor complex which is composed of the α, β, and γ subunits [18,19,26]. Recent evidence supports a more complex structure that in-

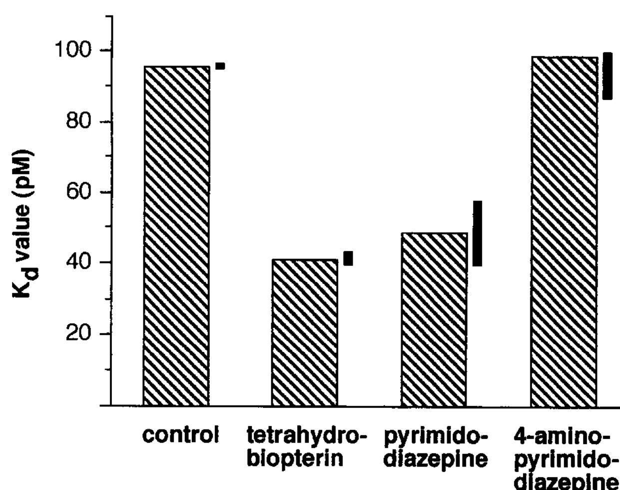

Fig. 1. Modulation of the interleukin 2 receptor affinity of MT-2 cells for the ligand. Tetrahydrobiopterin, (6*R*)-5,6,7,8-tetrahydrobiopterin (1); pyrimidodiazepine, 6-acetyl-2-amino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepin-4(3*H*)-one (2); 4-aminopyrimidodiazepine, 6-acetyl-2,4-diamino-7,8-dihydro-9*H*-pyrimido[4,5-b][1,4]diazepine (3).

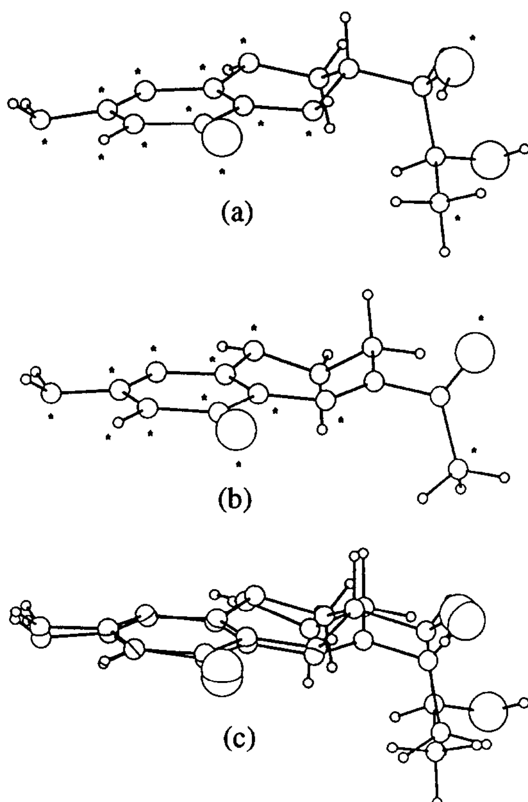

Fig. 2. Hypothetical active conformations of tetrahydrobiopterin (1) and pyrimidodiazepine (2), shown separately in (a) and (b) respectively, and superimposed in (c).

volves additional proteins such as the intercellular adhesion molecule-1 (ICAM-1) or the class I HLA molecule [19]. Available evidence suggests that the binding sites for tetrahydrobiopterin (1), and presumably also for pyrimidodiazepine (2), are located on the class I HLA molecules [27]. In an earlier study [11], semi-empirical molecular orbital (SMO) calculations on tetrahydrobiopterin (1) showed that while the bicyclic pteridine nucleus is structurally well-defined, the side chain at position 6 can occupy pseudoaxial or pseudoequatorial orientations. The side chain is also quite flexible, such that the molecule has at least eight energy minima within 1.5 kcal/mol of baseline. (Other recent calculations [28] may not have revealed the global minimum energy conformation, since a limited number of starting points was used.) Because of the flexibility of the side chain, any inferences which can be drawn from the calculations concerning the binding site of tetrahydrobiopterin (1) are limited. On the other hand, the pyrimidodiazepine (2) has less conformational freedom than (1) and, superficially at least, a quite different structure. It might be expected that a survey of its conformational space would reveal only a few points of intersection with (1), thus providing clear guidance as to the receptor structure. Indeed, a process of structure matching using configurations generated by SMO theory revealed very few pairs of conformations which showed reasonable correspondence. There was one pair which seemed outstanding, however, as illustrated in (a) and (b) in Fig. 2. Tetrahydrobiopterin (1) in the conformation shown in (a) is only 0.23 $kcal \cdot mol^{-1}$ above its global minimum as located in our earlier study [11]. Pyrimidodiazepine (2) in the conformation shown in (b) has $\theta = 70.8°$, and is thus very close to one of its energy minima. After superposition of the atoms marked with asterisks in the pyrimidine nuclei, the two side-chains were found to occupy much the same region of space, and the acetyl oxygen in the pyrimidodiazepine was found to lie within 0.22 Å of the C1′ oxygen in tetrahydrobiopterin. This is illustrated in (c), which shows conformations (a) and (b), of (1) and (2), respectively, superposed. Both conformations (a) and (b) are among the lowest-energy conformations of their respective molecules, and the conclusion that the compounds are bound to the receptor in these forms is, at least, a good working hypothesis. If correct, it may help direct research towards the development of small-molecule immunostimulants with therapeutic applications.

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