BORONIC ACID INHIBITORS OF[†] DIPEPTIDYL PEPTIDASE IV:

A NEW CLASS OF IMMUNOSUPPRESSIVE AGENTS

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[†] This chapter is dedicated to the memory of Dr. Simon Coutts.

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I. INTRODUCTION

The aim of this review is to discuss the design and development of a new class of immunosuppresive agents, which are inhibitors of the serine protease dipeptidyl peptidase IV (DPP IV). In particular, it will focus on the most potent class of inhibitors of this enzyme, dipeptides of proline boronic acid, which have been studied extensively in a collaborative effort between groups at Tufts University and at Boehringer Ingelheim.

II. DIPEPTIDYL PEPTIDASE IV AS A TARGET FOR IMMUNOSUPPRESSION

Dipeptidyl peptidase IV[1] is a membrane-bound enzyme found on the surface of a wide variety of cell types, including liver, kidney, and intestine.[2] It acts by cleaving a dipeptide from the free N-terminus of a polypeptide where the second residue is proline. Its function in these tissues is generally thought to be involved in the catabolism of proline-containing peptides.[3] DPP IV is also found on the surface of T-lymphocytes; its expression correlates with a memory phenotype, and it is highly expressed on double-negative thymocytes and natural killer cells.[4] In the mid-1980s several groups showed that the amount of DPP IV on lymphocytes increased upon activation.[5] Schön et al. subsequently showed that inhibitors of the enzyme block the proliferative response of T-cells to antigenic stimulation[6] and suppress IL-2 production.[7] DPP IV was later shown to be identical to the T-cell marker antigen known as CD26,[8,9] which had been identified as a costimulatory molecule in T-cell activation.[10] Cross-linking of CD26 with antibodies can potentiate the reponse to suboptimal stimulation of the T-cell receptor by anti-CD3. Recent reviews have appeared covering biochemical[11] and immunological[12] aspects of DPP IV. This review will focus on inhibitor design.

Details of the involvement of DPP IV in T-cell activation have not yet been elucidated. Early on it was noted that several cytokines, including IL-1β, IL-2, TNF-β, and GM-CSF, have N-terminal sequences that should be substrates for DPP IV. Hoffmann[13] has shown that short

peptides corresponding to the N-terminal sequence of these molecules are cleaved by the enzyme, but the full-length cytokines are not. Furthermore, short N-terminal deletions of either IL-1β[14] or IL-2[15] have little effect on their biological activity, arguing against this as a possible mechanism. To date there are no convincing candidates, either for the endogenous substrate, or for a possible ligand, such as another cell surface molecule. DPP IV has been reported to be associated both with CD45,[16] a tyrosine phosphatase known to be essential for T-cell activation, and with adenosine deaminase.[17] Mutations in adenosine deaminase result in a form of severe combined immune deficiency, but the majority of the enzyme is normally found in the cytosol, and the function of the membrane-associated form is not yet clear. Inhibitors of DPP IV have no effect on the association of ADA. Antibodies against CD26 induce tyrosine phosphorylation.[18,19] The human immunodeficiency virus (HIV) tat protein has been shown to bind to DPP IV and to inhibit its catalytic activity[20]; this may be involved in the pathogenesis of the virus. DPP IV was also reported to function as a receptor for infection of cells by HIV,[21] although other groups have presented data that cast doubt on this observation.[22] More work is required to elucidate the mechanism of action of DPP IV, but the effectiveness of DPP IV inhibitors in blocking proliferation, which is discussed in more detail below, indicates that it plays an important role in T-cell activation.

DPP IV is a 110-kDa homodimeric cell surface glycoprotein, with an intracellular N-terminus and a cytoplasmic domain of only six residues. Highly homologous cDNAs have been cloned from rat,[23] mouse,[24] and human cells,[25] which code for proteins of 767, 760, and 766 amino acids, respectively. The active site serine has been identified as Ser631 in the rat sequence[26] and Ser624 in mouse. In mouse, Asp702 and His734 have been shown to be the other members of the catalytic triad.[27] Based on this, Ser630, Asp702, and His740 comprise the catalytic triad of the human enzyme. DPP IV is not related to the classical serine proteases such as chymotrypsin, in either sequence or arrangement of catalytic residues, but is more similar to a family of peptidases and lipases,[28] and in particular to prolyl endopeptidase (PEP).[29] There has been some debate over whether the catalytic activity of DPP IV is essential for its function; antibodies against DPP IV such as 1F7, which block T-cell activation,[10c] have no effect on its enzymatic activity. Morimoto[30] has reported mutagenesis studies in which the active-site serine was replaced by alanine, which reduced the costimulatory activity of the molecule. Hegen,[31] studying CD26-transfected Jurkat cells, saw little effect on IL-2

production or proliferation with reversible inhibitors and concluded that enzyme inhibition has no effect. It is possible that more complete, or irreversible, inhibition is required to block the response. Recently it was observed that addition of a soluble form of DPP IV to cell cultures could potentiate the response to antigen,[32] but no effect was seen when the DPP IV was first inactivated with the irreversible inhibitor DFP. Although details of the mechanism of action of DPP IV in T-cell activation have still to be elucidated, the ability of inhibitors to block this response makes it an attractive target for therapeutic intervention.

III. SUBSTRATE SPECIFICITY OF DPP IV

Most of the information about the steric requirements of the active site has come from studies on artificial substrates, in most cases dipeptide *p*-nitroanilides.[11,33–35] These are reactive amides, and may not reflect the substrate preference of peptides. These studies showed that there is an absolute requirement for a free N-terminus (P_2 site, Fig. 1). There is a strong preference for proline at the second position (P_1 site), but artificial substrates with hydroxyproline or alanine are also cleaved, between 100- and 1000-fold less efficiently, as judged by comparing k_{cat}/K_m. The five-membered ring of proline can be unsaturated, or contain an O or S atom without losing activity.[34] The azetidine analog is a slightly better substrate, and piperidine is about fivefold poorer than proline.[34] Almost any amino acid can occupy the terminal position (P_2).[33c,35] There is a slight preference for hydrophobic side chains, but among natural amino acids there is only about a 10-fold

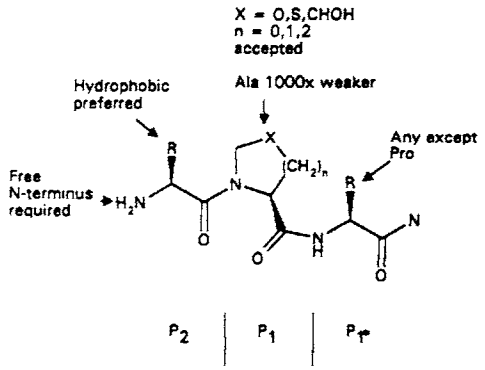
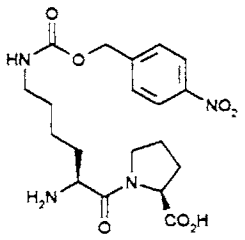


Figure 1. Substrate specificity of DPP IV.

difference between the best and the poorest substrate. Monomethylation of the amine is tolerated in Sar-Pro, but further methylation abolishes activity. The enzyme has been shown to recognize the *trans* form of the H-Xaa-Pro peptide bond.[36] The P_1 residue can be anything except a second proline. As might be expected, peptides containing D-amino acids are not cleaved.

IV. INHIBITORS OF DPP IV

The first inhibitors of DPP IV to be described were the LysPro derivative **1** and the acyl hydroxamic acid **2**.[5b] These were not particularly potent, but **2**, which is an irreversible inhibitor, with K_i of 30 μM and k_{inact} of $6.2 \times 10^{-4} s^{-1}$, was the first compound used to demonstrate blockade of T-cell function by DPP IV inhibition. The mechanism of inhibition of DPP IV by **2** and its analogs has been studied in some detail.[37,38] The acyl hydroxamic acids are suicide substrates, which are converted to a reactive intermediate, probably an isocyanate, in the active site. However, they are cleaved by the enzyme more efficiently than they are activated to generate the reactive species, which is the reason for their modest potency. Compound **1** reflects the inherently limited affinity of a product-based inhibitor. The tripeptides diprotin A and B were reported to be inhibitors[39] but in fact are poor substrates.[40] Azapeptide esters such as **3** have been shown to inactivate DPP IV at 20–100 μM,[41] presumably by forming a stable acyl enzyme intermediate. Reactivation is quite rapid, however, and the compounds are chemically unstable in solution at pH



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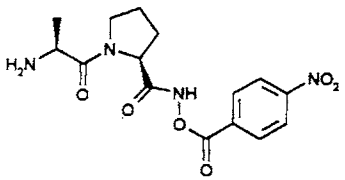

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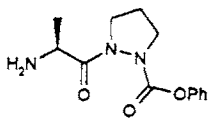

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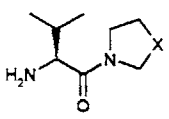

4 X = CH_2, S

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