# Specificity of *Neisseria catarrhalis* Arylamidase[1] (34662)

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*Neisseria catarrhalis* produces an intracellular enzyme which catalyzes the hydrolysis of certain neutral amino acid derivatives of β-naphthylamine (βNA) and structurally related polypeptides. We have previously reported on the purification and general properties of this bacterial enzyme, arylamidase-I, and on some of the essential features of substrate structure in relation to substrate susceptibility to hydrolysis by this enzyme. The term, arylamidase, has gained wide usage in the literaure and we have utilized it in the same sense as other investigators have, pending a definitive elucidation of its role.

Generally, the rate of hydrolysis of amino acid-βNA is greatest when the amino acid residue is alanine even though a number of other neutral, non-beta branched amino acid-βNA derivatives are also cleaved at significant rates. The hydrolysis of dipeptide-βNA occurs in a stepwise manner, thus suggesting that arylamidase functions as an exopeptidase (1). This hypothesis was supported by experiments in which the initial hydrolytic products of L-alanyl-L-alanine-*βNA* were shown to be L-alanine plus L-alanine-βNA, the latter was subsequently cleaved to yield a second mole of alanine and βNA. L-Alanyl-D-alanine-βNA yielded L-alanine and D-alanine-βNA, a product which was totally resistant to further hydrolysis.

The data presented here established the manner in which this enzyme acts on alanine polypeptides and establish the essential features of substrate structure required by arylamidase.

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*Materials and Methods.* Substrates were obtained from Mann Research Laboratories, New York, N.Y., and from the International Chemical and Nuclear Co., Burbank, California. The fluorimetric and colorimetric procedures for arylamidase assay and a paper chromatographic method for amino acid and polypeptide identification were the same as those previously reported (2). The concentration of amino acid in reaction mixtures was estimated by comparing the size and intensity of the spot produced by an unknown amount of a certain amino acid with the size and intensity of spots produced by known amounts of the same amino acid similarly chromatographed.

*Results and Discussion.* Only alanine peptides of uniform L-configuration were completely hydrolyzed, *e.g.* (L-alanine)$_2$, (L-alanine)$_3$, (L-alanine)$_4$, and (L-alanine)$_5$. L-Alanyl-L-alanyl-D-alanine was hydrolyzed to yield L-alanine and L-alanyl-D-alanine; on the other hand, L-alanyl-D-alanyl-D-alanine was completely resistant to hydrolysis. This indicates that the penultimate residue must also be of the L-configuration if hydrolysis is to occur. A *d*-isomer not in the N-terminal or penultimate position apparently has little or no effect on the susceptibility of peptides to arylamidase catalyzed hydrolysis. Additional chromatographic studies of reaction mixtures containing (L-alanine)$_3$, (L-alanine)$_4$, or (L-alanine)$_5$ showed that the first hydrolytic products formed were the corresponding peptide one residue shorter and alanine. Of course in subsequent aliquots withdrawn from the reaction mixture, all the other successively shortened peptides were also identifiable and finally after longer incubation only alanine appears. These results are shown on Fig. 1. Activity on other alanine peptides depends on the position of the D-isomer, if

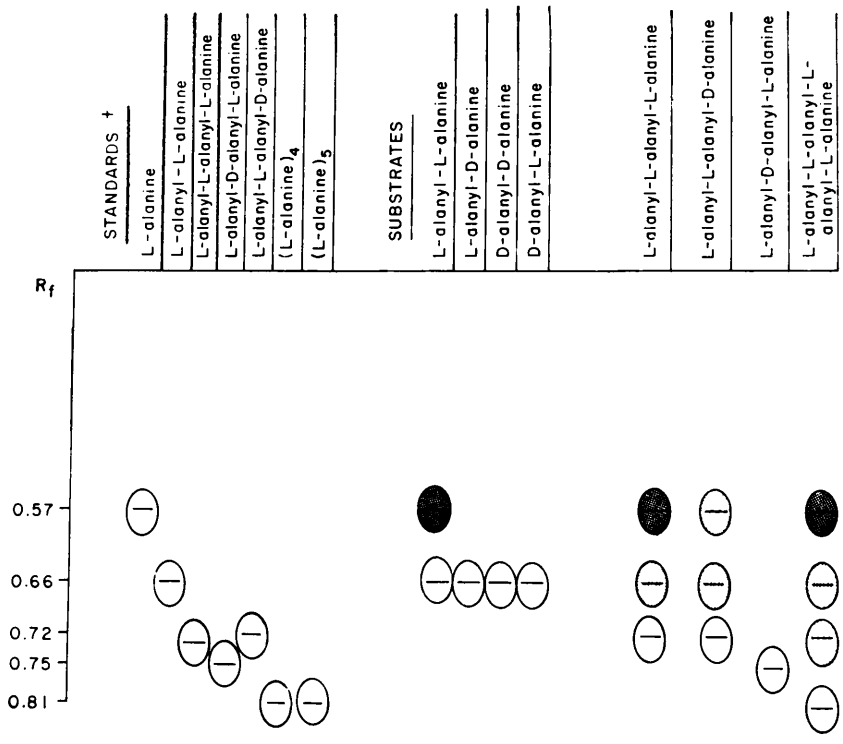


FIG. 1. Composite chromatogram showing the products of arylamidase catalyzed hydrolysis of alanine peptides. Samples from each reaction mixture (containing 2 $\mu$moles of substrate/ml plus enzyme in 0.01 *M* phosphate buffer) were inactivated at 5-min intervals during the incubation period, and were chromatographed at room temperature in water saturated phenol. Zero-time reaction mixtures gave spots only at $R_f$ values corresponding to authentic substrates. Shaded spots indicate the relative amounts and "build-up" with time of the various products in the reaction mixture (see text). The results with penta-alanine are not depicted since the $R_f$ for it and tetra-alanine are the same in the solvent system used here.

present. In general, however, our finding concerning the steroisomers of alanine and the positioning of these isomers agree closely with Schechter's findings for LAP (3), who also suggested that exopeptidases and other proteolytic enzymes are able to recognize certain sequences and sizes of amino acid residues (4).

Next, the susceptibilities of two C-terminal substituted derivatives of alanylalanine to arylamidase catalyzed hydrolysis were determined, again by paper chromatographic studies of the composition of reaction mixtures. In the first of these experiments the substrate utilized was L-alanyl-L-alanineamide. Studies of aliquots of the reaction mixture taken after various time intervals showed that alanine and alanineamide rapidly appeared as reaction products whereas the latter was very slowly hydrolyzed. In the second set of experiments L-alanyl-L-alanine methyl ester was employed as the substrate; the same general results were obtained with the products of hydrolysis being alanine and alanine methyl ester, which was resistant to further hydrolysis.

In the next experiments the susceptibilities to arylamidase catalyzed hydrolysis of several substrates with modifications or substitution involving the N-terminal amino group were determined fluorometrically. Propionic acid-$\beta$NA, lactic acid-$\beta$NA, formic acid-$\beta$NA, $\beta$-alanine-$\beta$NA, *N*-acetyl-alanine-$\beta$NA, and D-alanine-$\beta$NA, were totally resistant to hydrolysis by arylamidase. These results indicate an absolute requirement for an

unsubstituted α-amino group of the L-configuration. Other reports from this laboratory have dealt with the relationship between the size and branch point of the R-group of the N-terminal residue and the binding and hydrolysis of substrates by arylamidase (1, 5).

It appears then that arylamidase catalyzed hydrolysis of certain peptides is a stepwise process, beginning with the N-terminal residue and continuing to completion provided the initial and succeeding N-terminal residues have a free α-amino group and are of the L-configuration, and provided the corresponding penultimate residues are also of the L-configuration.

The stepwise nature of the mechanism of hydrolysis was also indicated by kinetic studies of the hydrolysis of L-alanine-βNA vis-a-vis L-alanyl-L-alanine-βNA, the former being characterized by a rapid and linear appearance of βNA, while the latter exhibited an initial lag in βNA production. On the other hand, if an intact dipeptide had been released from the latter substrate there would have been no lag in the appearance of βNA (1).

These results, therefore, establish the exopeptidase activity of this enzyme. The physiological importance of this enzyme remains unclear; however, in view of the usefulness of exopeptidases in the determination of N-terminal amino acid residues, and in view of the specific substrate requirements of this enzyme, its application to protein chemistry is of possible importance.

*Summary.* Bacterial arylamidase has an absolute requirement for an unsubstituted α-amino group of the L-configuration on the N-terminal residue of substrates hydrolyzed by this enzyme. The penultimate residue must also be of the L-configuration. This enzyme acts as an exopeptidase beginning at the N-terminal residue and cleaving one residue at a time.

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