From:
The Institute of Dentistry,
University of Turku,
Finland

SELECTIVE EFFECT OF QUATERNARY AMMONIUM SALTS ON THE ACTIVITY OF ARYLAMINOPEPTIDASES OF THE DENTAL PULP, AND ON TRYPSIN, CHYMOTRYPSIN AND LEUCINE AMINOPEPTIDASE

by
KAUKO K. MÄKINEN
RISTO BRUMMER
ARJE SCHEININ

INTRODUCTION

Several surface active agents have been used in medicine because of their bactericidal activity. The mode of their action in affecting proteins and killing bacteria has been earlier reviewed (*Hotchkiss*, 1946; *Putnam*, 1948; *Valko*, 1946; *Price*, 1946; *Putnam & Neurath*, 1944; *Glassman*, 1948). Recently, some cationic surfactants have been investigated for their selective effect on some hydrolytic enzymes (*Mäkinen*, 1968; *Mäkinen & Mäkinen*, 1970). These compounds — benzyldimethyl {2- [2- (p-1, 1, 3, 3-tetramethylphenoxy) ethoxy]-ethyl}ammonium chloride (benzethonium chloride) and dodecyldimethyl(2-phenoxyethyl)ammonium bromide (domiphen bromide) — inhibited some proline iminopeptidases and arylaminopeptidase-like enzymes of human whole saliva, while the enzymic hydrolysis of p-nitrop henyl phosphate by enzyme preparations derived from certain other sources and the chymotrypsin-catalyzed reactions were seen to be activated by domiphen bromide. Efforts to trace the mechanism of these effects have been made earlier (*Mäkinen & Mäkinen*, 1970).

The purpose of this paper is to summarize the most important results of experiments made to elucidate the effect of dequalinium chloride (decamethylenebis[4-aminoquinaldinium chloride]), cetylpyridinium chloride (1-hexadecylpyridinium chloride), benzethonium chloride, benzalkonium

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chloride (a mixture of alkyldimethylbenzylammonium chlorides where the alkyls range from C_8H_{17} to $C_{18}H_{37}$), and domiphen bromide on the activity of arylaminopeptidase-like enzymes derived from swine dental pulp. For comparison, data from experiments with swine serum, red cell enzyme preparations, and certain commercial enzymes were included.

MATERIALS AND METHODS

All of the materials and methods were the same as earlier described (*Mäkinen, Brummer & Scheinin*, 1970; *Mäkinen & Mäkinen*, 1970). The phosphatase activity with p-nitrophenyl phosphate was, in principle, assayed according to *Bessey, Lowry* and *Brock* (1946).

RESULTS

It was first found necessary to study the effect of certain quaternary ammonium salts on the hydrolysis of *N*-L-aminoacyl-2-naphthylamines by crude swine pulp enzyme preparations. Results are shown in Table I which indicate that the quaternary ammonium salts with short side chains had no marked effect on the rate of the enzymic reactions, while those with longer alkyl chains displayed considerable inhibition. The same Table also presents results from experiments with the partially purified aminopeptidase B-like enzyme of swine pulp and from experiments with p-nitrophenyl phosphate as substrate. With the latter substrate the quaternary ammonium salts caused strong activation. This is more illustratively shown in Fig. 1 for quaternary ammonium salts with longer alkyl chains.

Fig. 2 shows the effect of dequalinium chloride on the hydrolysis of some *N*-L-aminoacyl-2-naphthylamines by arylaminopeptidase-like enzymes derived from swine serum and red blood cells. The enzymes derived from swine dental pulp yielded essentially similar results. The quaternary ammonium compounds strongly inhibited all aminopeptidase-like enzymes regardless of their source. All the experiments presented in Fig. 2 (and in Fig. 5) were carried out at several substrate concentrations. Because no reliable value of the inhibition constant, K_i, could be determined by graphical means (due to the curvature), results with only two substrate concentrations are shown.

Since the properties and mechanism of action of certain commercial enzymes is better known than those of any of the pulpal enzymes investigated in this study, it was thought useful to test the effect of some of the quaternary ammonium salts on the activity of trypsin, chymotrypsin and leucine

Table I

Effect of some quaternary ammonium salts on the rate (in $10^7 \times M\ min^{-1}$) of the hydrolysis of some N-L-aminoacyl-2-naphthylamines and p-nitrophenyl phosphate by enzyme preparations derived from swine dental pulp. The effect can be compared to that obtained without added affector (water instead). Substrate concentration: 0.166 mM. The arylaminopeptidase activity was tested in 0.025 M phosphate buffer, pH 7.2, in the presence of 0.2 M NaCl, and the phosphatase activity was tested in 0.05 M tris-HCl buffer, pH 7.2. The enzyme preparations were made as previously described (Mäkinen, Brummer & Scheinin, 1970).

Compound	Unfractionated enzyme preparation from swine dental pulp			Partially purified aminopeptidase B-like enzyme
	N-L-Arginyl-2-NA	*N*-L-Methionyl-2-NA	p-Nitrophenyl phosphate	*N*-L-Arginyl-2-NA
Tetra-*N*-methylammonium iodide	0.61	0.54	0.47	1.43
Tetra-*N*-butylammonium iodide	0.54	0.52	0.38	1.49
Tetra-*N*-butylammonium chloride	0.47	0.53	0.55	1.51
Domiphen bromide	0.12	0.21	1.27	0.05
Benzethonium chloride	0.30	0.40	1.58	0.04
Cetylpyridinium chloride	0.23	0.19	1.06	<0.01
Dequalinium chloride	0.51	0.22	1.52	0.05
H_2O	0.54	0.60	0.47	1.48

aminopeptidase. Results from these experiments are shown in Figs. 3—5. Dequalinium chloride increased the trypsin-catalyzed hydrolysis of *N*-L-benzoylarginyl-2-napthylamine when the concentration of the compound needed for maximal activation depended on substrate concentration (Fig. 3).

Fig. 4 demonstrates that with each of the ammonium compounds investigated the maximum activation of chymotrypsin was attained at different concentrations when the hydrolysis of *N*-L-methionyl-2-naphthylamine was studied. The same result was obtained with *N*-L-leucyl-2-naphthylamine as well. From Fig. 5 it is evident that cetylpyridinium chloride potently inhibited the leucine aminopeptidase-catalyzed hydrolyses. In the trypsin-catalyzed hydrolysis of *N*-L-benzoylarginyl-2-naphtylamine the effect of cetylpyridinium chloride was clearly different from that exerted by other quaternary ammonium salts (Fig. 5 B).

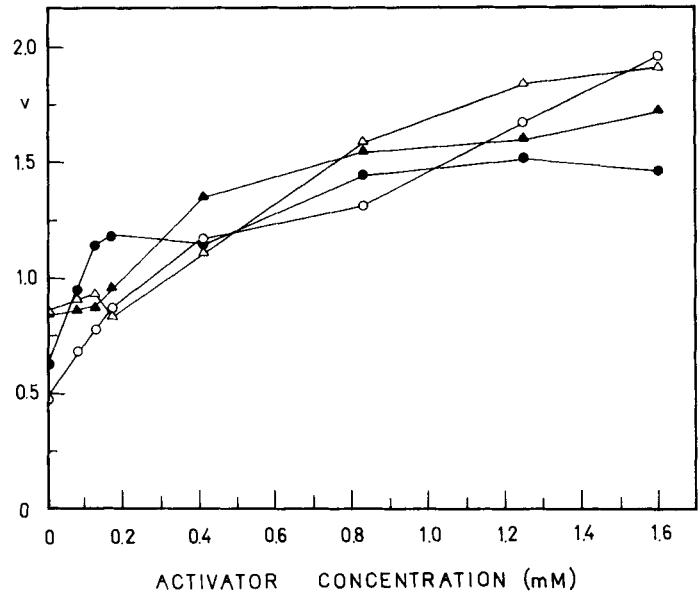


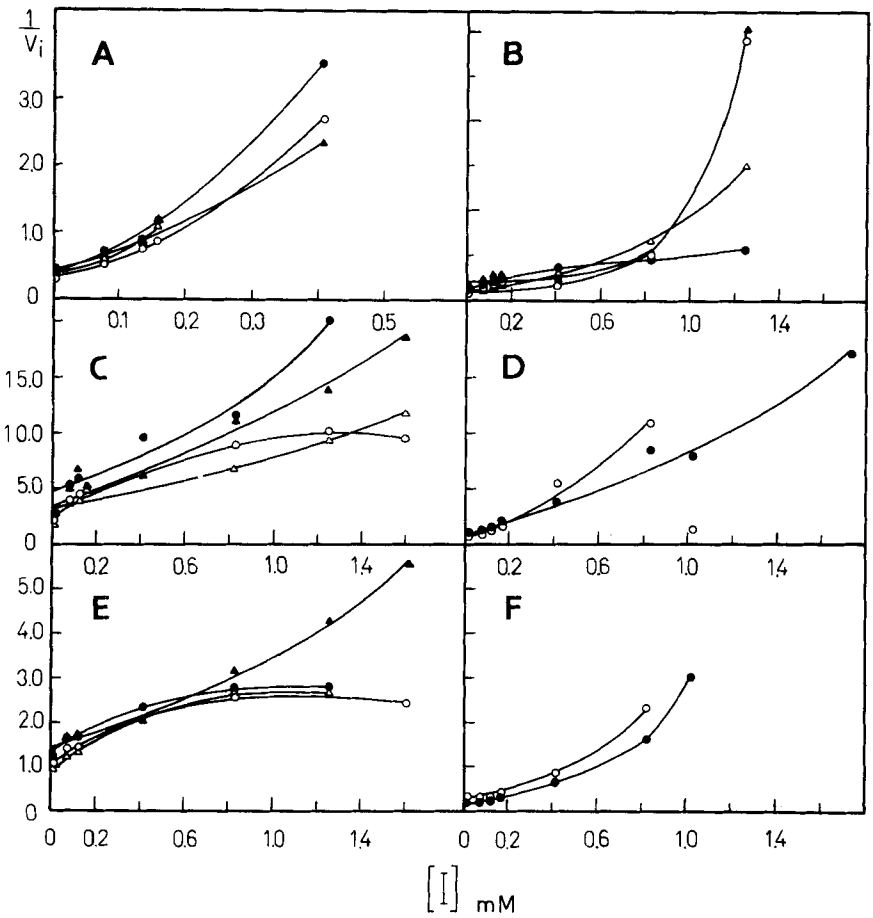
Fig. 1. Effect of some quaternary cation active ammonium salts on the rate v (in $10^7 \times$ M min^{-1}) of the hydrolysis of p-nitrophenyl phosphate by an enzyme preparation obtained from swine pulp homogenate. O—O, dequalinium chloride; ●—●, cetylpyridinium chloride; △—△, benzethonium chloride; ▲—▲, domiphen bromide.

Fig. 2. Inhibition by dequalinium chloride of several arylaminopeptidase-catalyzed hydrolyses of some *N*-L-aminoacyl-2-naphthylamines (tested at two substrate concentrations).

A. ●—●, Hydrolysis of *N*-L-alanyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool I from swine serum.
O—O, Hydrolysis of *N*-L-alanyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool II from swine serum.
▲—▲, Hydrolysis of *N*-L-alanyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool I from swine serum.
△—△, Hydrolysis of *N*-L-alanyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool II fron swine serum.

B. ▲—▲, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool I from swine serum.
O—O, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool II from swine serum.
△—△, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool II from swine serum.
●—●, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool I from swine serum.

C. ●—●, Hydrolysis of *N*-L-leucyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool I from swine serum.
▲—▲, Hydrolysis of *N*-L-leucyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool I from swine serum.
O—O, Hydrolysis of *N*-L-leucyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool II from swine serum.
△—△, Hydrolysis of *N*-L-leucyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool II from swine serum.



D. ●—●, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.333 mM) by a DEAE cellulose pool from swine erythrocytes (=aminopeptidase B preparation).
O—O, Hydrolysis of *N*-L-lysyl-2-naphthylamine (0.166 mM) by a DEAE cellulose pool from swine erythrocytes (=aminopeptidase B preparation).

E. ▲—▲, Hydrolysis of *N*-L-methionyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool I from swine serum.
●—●, Hydrolysis of *N*-L-methionyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool I from swine serum.
△—△, Hydrolysis of *N*-L-methionyl-2-naphthylamine (0.333 mM) by DEAE cellulose pool II from swine serum.
O—O, Hydrolysis of *N*-L-methionyl-2-naphthylamine (0.166 mM) by DEAE cellulose pool II from swine serum.

F. O—O, Hydrolysis of *N*-L-arginyl-2-naphthylamine (0.166 mM) by a DEAE cellulose pool from swine erythrocytes (=aminopeptidase B preparation).
●—●, Hydrolysis of *N*-L-arginyl-2-naphthylamine (0.333 mM) by a DEAE cellulose pool from swine erythrocytes (=aminopeptidase B preparation).
The DEAE cellulose pools were obtained as described in the previous paper (*Mäkinen, Brummer & Scheinin*, 1970).

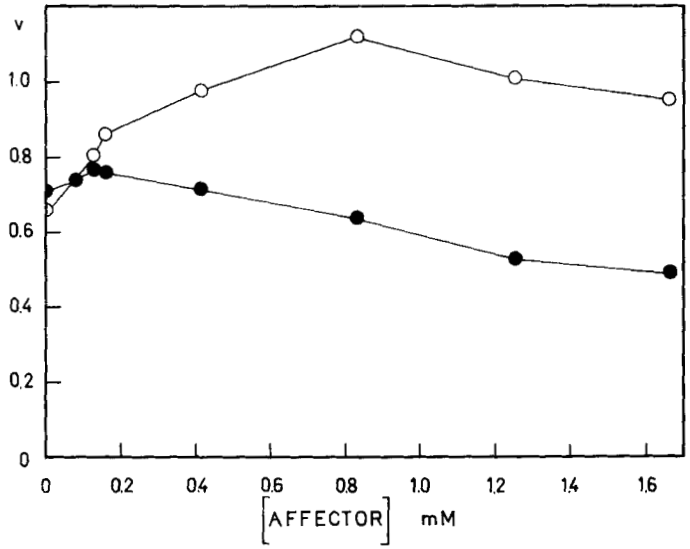


Fig. 3. Effect of dequalinium chloride on the rate v (in $10^7 \times$ M min^{-1}) of the hydrolysis of *N*-L-benzoylarginyl-2-naphthylamine by trypsin. Substrate concentrations: O—O, 0.333×10^{-3} M; ●—●, 0.116×10^{-3} M.

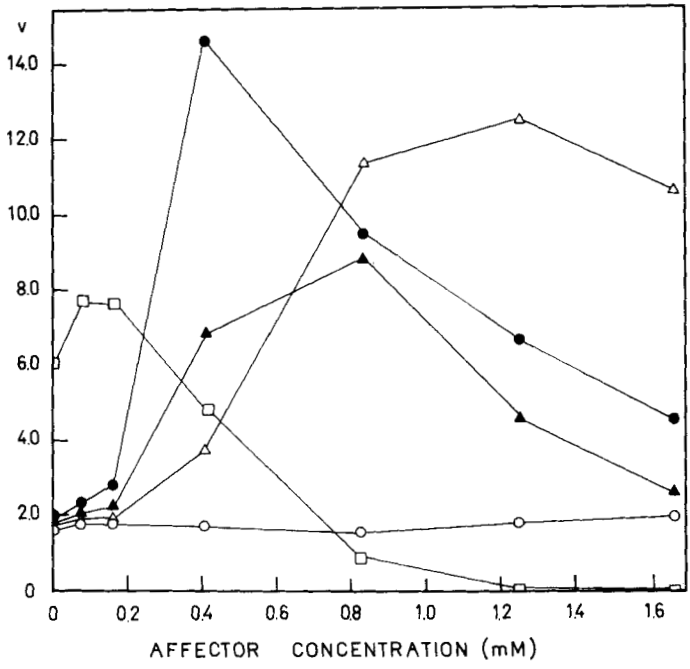


Fig. 4. Effect of some quaternary cation active ammonium salts on the rate v (in $10^7 \times$ M min^{-1}) of the hydrolysis of *N*-L-methionyl-2-naphthylamine by chymotrypsin. ●—●, domiphen bromide; △—△, benzalkonium chloride; ▲—▲, benzethonium chloride; O—O, dequalinium chloride; □—□, cetylpyridinium chloride. Tested at 0.166×10^{-3} M substrate concentration.

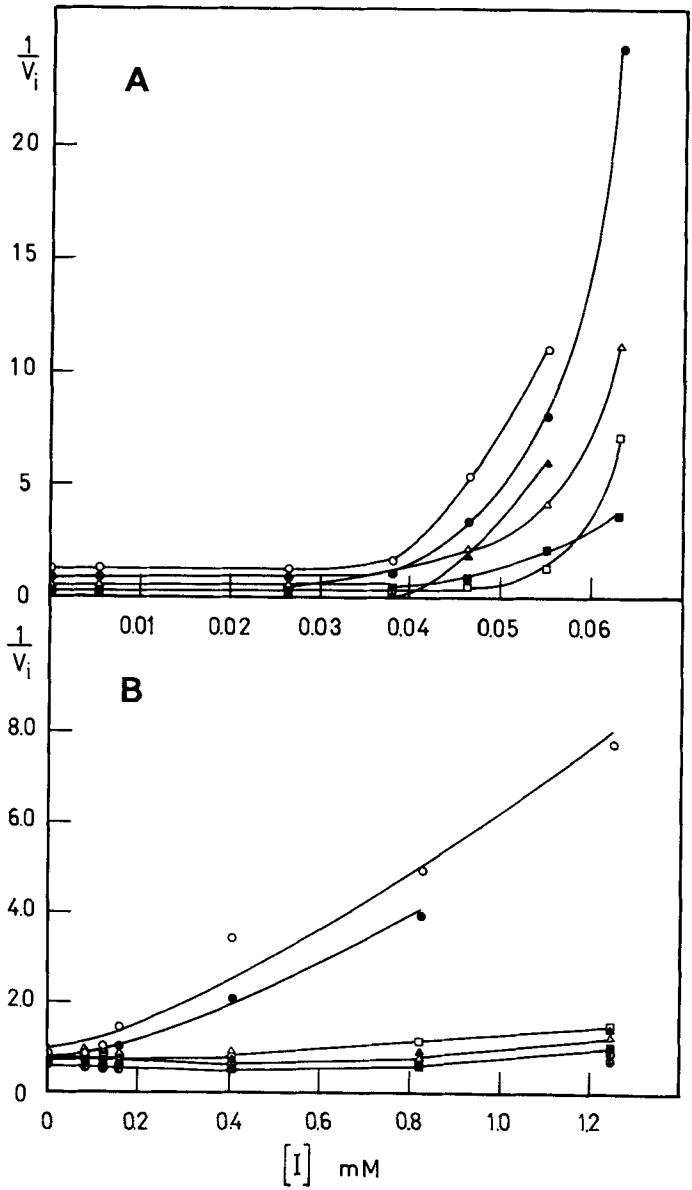


Fig. 5. Dixon plots of the inverse value of v_i against the concentration of the inhibitor, [I], in the hydrolysis of some *N*-L-aminoacyl-2-naphthylamines catalyzed by leucine aminopeptidase and inhibited by cetylpyridinium chloride (A), and in the trypsin-catalyzed hydrolysis of *N*-L-benzolylarginyl-2-naphthylamine, inhibited by several quaternary ammonium salts (B). Results with two substrate concentrations are given. Legend for A: O—O, *N*-L-leucyl-2-naphthylamine (0.166×10^{-3} M); ●—●, *N*-L-leucyl-2-naphthylamine (0.333×10^{-3} M); △—△, *N*-L-alanyl-2-naphthylamine (0.166×10^{-3} M); ▲—▲, *N*-L-phenylanyl-2-naphthylamine (0.166×10^{-1} M); ■—■, *N*-L-alanyl-2-naphthylamine (0.333×10^{-3} M); □—□, *N*-L-phenylalanyl-2-naphthylamine (0.333×10^{-3} M). Legend for B (hydrolysis of *N*-L-benzoylarginyl-2-naphthylamine): O—O, [S]: 0.166×10^{-3} M, inhibitor: cetylpyridinium chloride; ●—●, [S]: 0.333×10^{-3} M, inhibitor: cetylpyridinium chloride; ■—■ and □—□, as above but the inhibitor was benzethonium chloride; △—△ and ▲—▲, as above, but the inhibitor was domiphen bromide; ⊗—⊗ and ◑—◑ (the lowest experimental points), as above, but the inhibitor was benzalkonium chloride.

DISCUSSION

The pattern of enzyme inhibition found in this paper greately resembled that elsewhere observed with aminopeptidase-like enzymes (*Mäkinen & Mäkinen*, 1970) i.e. the aminopeptidase-like enzymes were inhibited when the inverse value of reaction rate (in the Dixon plots) did not increase linearly with inhibitor concentration. The mechanism of this kind of enzyme inhibition has been considered elsewhere (*Mäkinen & Mäkinen*, 1970).

The results showed that the quaternary ammonium compounds with long alkyl chains displayed diverse effects depending on the nature of the enzymic reaction. When cells of the pulp or other dental tissue were placed in contact with dequalinium chloride, for example, the pulpal aminopeptidase-like enzymes were strongly inhibited, while phosphatase-like enzymes were activated. This selective effect may offer an explanation for the effects exerted by dequalinium chloride in endodontic treatment. The so called organic »aminfluorides», used among others by *Mühleman* (1967) as caries preventive agents, are by their structure related to those compounds employed in this study. Also their effect could be explained on the basis earlier discussed (*Mäkinen & Mäkinen*, 1970). The use of the dequalinium cation crystallized as fluoride instead of chloride, would extend its field of application.

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SUMMARY

The effect of some quaternary cation active ammonium salts on the hydrolysis of some *N*-L-aminoacyl-2-naphthylamines and p-nitrophenyl phosphate by enzyme preparations derived from swine dental pulp, red cells, and erythrocytes was studied. For comparison, the effect of the same compounds on the trypsin-, chymotrypsin- and leucine aminopeptidase-catalyzed reactions was tested. The quaternary ammonium salts included domiphen bromide, benzethonium chloride, cetylpyridinium chloride and dequalinium chloride. The compounds strongly inhibited all aminopeptidase-like enzymes, including partially purified aminopeptidase B of the pulp, while the rate of the reactions catalyzed by trypsin, chymotrypsin and a phosphatase-like enzyme generally increased in the presence of these compounds. The enzymic hydrolysis of p-nitrophenyl phosphate by pulpal enzymes was increased as much as threefold by quaternary ammonium salts with long alkyl chains.

RESUMÉ

ACTION SÉLECTIVE DES SELS D'AMMONIUM QUATERNAIRE SUR L'ACTIVITÉ DES ARYLAMINOPEPTIDASES DE LA PULPE DENTAIRE, ET SUR LA TRYPSINE, LA CHYMOTRYPSINE ET LA LEUCINE-AMINOPEPTIDASE

Une étude a été effectuée sur l'action de quelques sels cationiques d'ammonium quaternaire sur l'hydrolyse de quelques *N*-L-aminoacyl-2-naphtylamines et du p-nitrophénylphosphate par des préparations enzymatiques provenant de la pulpe dentaire, des globules rouges et des érythrocytes du porc. Á titre de comparaison, des expériences ont été faites sur l'action des mêmes produits sur les réactions catalysées par la trypsine, la chymotrypsine et la leucine-aminopeptidase. Les sels d'ammonium comprenaient le bromure de domiphène, le chlorure de benzéthonium, le chlorure de cétylpyridinium et le chlorure de déqualinium. Ces produits inhibaient fortement tous les enzymes du type amidopeptidase, y compris l'amidopeptidase B partiellement purifiée provenant de la pulpe, tandis que les réactions catalysées par la trypsine, la chymotrypsine et un enzyme du type phosphatase étaient activées en présence de ces produits. L'hydrolyse enzymatique du p-nitrophénylphosphate par les enzymes pulpaires se trouvait augmentée jusqu'à trois fois par les sels d'ammonium quaternaire à longues chaines alkyl.

ZUSAMMENFASSUNG

DER SELEKTIVE EFFEKT QUATERNÄRER AMMONIUMSALZE AUF DIE AKTIVITÄT VON ARYLAMINOPEPTIDASEN DER ZAHNPULPA, UND AUF TRYPSIN, CHYMOTRYPSIN UND LEUCIN-AMINOPEPTIDASE

Es wurde der Effekt einiger quaternärer kationaktiver Ammoniumsalze auf die durch Enzympräparate, die aus der Schweinezahnpulpa und aus Erythrozyten gewonnen wurden, bewirkte Hydrolyse einiger *N*-L-Aminoacyl-2-Naphtylamine und p-Nitrophenyl untersucht. Zum Vergleich wurde die Wirkung der gleichen Verbindungen auf die durch Trypsin, Chymotrypsin und Leucin-Aminopeptidase katalysierten Reaktionen geprüft. Die quarternären Ammoniumsalze schlossen Domiphenbromid, Benzethonchlorid, Cetylpyridinchlorid und Dequalinchlorid ein. Die Verbindungen behinderten alle Aminopeptidase-ähnlichen Enzyme einschliesslich teilweise purifizierter Aminopeptidase B der Pulpa stark, während sich die Rate der durch Trypsin, Chymotrypsin und eines Phosphatase-ähnlichen Enzyms katalysierten Reaktionen in Gegenwart dieser Verbindungen erhöhte. Die enzymatische Hydrolyse von p-Nitrophenylphosphat durch Pulpenenzyme stieg etwa dreifach mittels quaternärer Ammoniumsalze mit langen Alkylketten.

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Address:
Institute of Dentistry,
University of Turku,
Turku 3, Finland