Vol. 115, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 15, 1983 Pages 632-641

# PURIFICATION AND CHARACTERIZATION OF ENKEPHALIN-DEGRADATING ENZYMES FROM CALF-BRAIN STRIATUM

**J.G.C. van Amsterdam, K.J.H. van Buuren and W. Soudijn**

Department of Pharmacy, Division of Pharmaceutical Chemistry
University of Amsterdam
Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

Received June 28, 1983

Enkephalinase A and B are extracted from Triton-X 100 washed calf-brain particles and purified by DEAE-cellulose chromatography. Both enzymes have identical $K_m$ values in their membrane-bound and soluble form.
Enkephalinase A has a pH optimum at 6.9 and a $K_m$ for Leu-enkephalin of 20-25 µM, which hardly depends on the pH. Thiorphan and phosphate are purely competitive inhibitors of Enkephalinase A with $K_i$ values of 3 nM and 1.5 mM respectively (pH = 6.85). Enkephalinase B is not affected by phosphate or thiorphan. It has a $K_m$ for Leu-enkephalin of 10 µM, a pH optimum of 7.0 and is inhibited by low concentrations of apolar dipeptides.

Met-enkephalin and Leu-enkephalin, the endogenous opioid pentapeptides are believed to play an important role in the transmission of nociceptive stimuli (1). After their release and action they are rapidly degraded ($t < 10$ s) most likely by a specific enzyme system (2). Many enzymes capable in degrading enkephalins are known (3-5), but the attention was mainly focussed on the soluble tyrosine producing aminopeptidases and the membrane-bound Enkephalinase A which produces tyrosyl-glycyl-glycine.

A third enkephalin-degrading enzyme, also present in brain is Enkephalinase B, a tyrosyl-glycine producing enzyme (6-10). The localization and characterization of such enzymes was hampered by the difficulties encountered in separating Tyr-Gly from Tyr-Gly-Gly and by the fact that this enzyme is present in the soluble fractions where huge amounts of aminopeptidase are present so that only tiny amounts of Tyr-Gly are produced. This apparent lack of Enkephalinase B activity led to the conclusion that only small amounts of this enzyme is present in the cells, and that it is of no importance for enkephalin biodegradation.

Using our improved HPLC technique we were able to demonstrate substantial amounts of Enkephalinase A and B activity in particulate preparations of both rat- and calf-brain striatum (8).

The present communication extends this observation and reports on the isolation and characterization of these two endopeptidases. Some of the results have been presented in a preliminary from (10).

0006-291X/83 $1.50

## METHODS AND MATERIALS

Isolation of enzymes. 10-16 Calf-brain striata were homogenized in 5-6 vol ice-cold Tris.HCl buffer (20 mM, pH 7.5) and centrifugated at 17 000 x g for 30 min. The pellet was washed twice by resuspension in 20-25 vol Tris.HCl buffer (17 000 x g for 30 min). The precipitate, was then mixed with 5-6 vol 0.1% Triton X 100 in Tris.HCl buffer and stirred at room temperature for 1 h. The clear supernatant obtained after centrifugation at 50 000 x g for 30 min is saved for the isolation of Enkephalinase B.

Enkephalinase A is extracted from the precipitate with 5-6 vol Tris.HCl buffer containing 1% Triton X 100 (Tris/Triton buffer). After stirring for 1 h at ambient temperatures the undissolved materials were sedimented by centrifugation at 50 000 g. The supernatant, filtered through 0.8 µm millipore disks, was then loaded on a DEAE-cellulose column (h=60 cm, $\phi$= 3.4 cm), pre-equilibrated with Tris/Triton buffer. After overnight washing with this buffer, Enkephalinase A was eluted with 0.1 M NaCl in Tris/Triton buffer (flow rate 35 ml/h). The fractions containing Enkephalinase A activity were pooled and concentrated by ultrafiltration.

Enkephalinase B is isolated from the 50 000 x g supernatants in a similar way, except that the column was washed with 0.2 M NaCl in Tris/Triton buffer and that the enzyme was eluted with 0.5 M NaCl in the same buffer. Sometimes the Enkephalinase B preparation was rechromatographed on a small column (h=15 cm, $\phi$= 1,5 cm) using a 0.1 - 0.5 M NaCl gradient to elute the enzyme.

The final preparations were stored in liquid $N_2$ in the form of small spheres with a volume of 15-20 µl. Unless otherwise stated, the temperature during the isolation was kept between 0 and 4°C.

Enzyme assay. The determination of the enzymic activity and the quantitation of the reaction products have been performed as described before (8). Enzyme activity was determined in the presence of 10 M [$^3$H] Leu-enkephalin (0,1 Ci/mmole), 1 mM puromycin and 1 µM captopril, unless otherwise stated.

Chemicals. [$^3$H]Leu-enkephalin (25 Ci/mmole) was obtained from New England Nuclear (Boston, U.S.A.). Small aliquots were purified regularly according to the method described by Vogel and Altstein (11). Leu-enkephalin, its degradation products and dipeptides were obtained from Serva (Heidelberg, G.F.R.). Puromycin and organic phosphates were from Sigma (St. Louis, U.S.A.) and Thiorphan was kindly donated by Duphar B.V. (Weesp, The Netherlands).

## RESULTS

1. Enzyme isolation.

Several enzymes present in the homogenate of calf-brain striatum hydrolyze Leu-enkephalin. This is shown in Table I and Fig. 1, which summarize the production of the metabolites of these enzymes (Tyr, Tyr-Gly and Tyr-Gly-Gly respectively) at various stages during the isolation of Enkephalinase A and B.

By far the largest activity in the homogenate is attributable to that of the aminopeptidase but by the addition of puromycin, an effective aminopeptidase inhibitor, it became clear that also appreciable amounts of Enkephalinase B are present in the crude homogenate. In contrast, the amount of Enkephalinase A activity is low. Aminopeptidase and Enkephalinase B are soluble enzymes: they do not precipitate at 280 000 x g for 2 h. Enkephalinase A is membrane bound, but subsequent washings (up to 6 times) with buffer could not remove the soluble enkephalin-degrading enzymes from the precipitates obtained by centrifugation at 17 000 x g for 30 min. Since dispersion and/or denaturation cause a rapid decrease in the total Enkephalinase A activity after 3-4 washings we washed our particles twice.

TABLE I

Distribution of Leu-enkephalin hydrolyzing enzymes at the different stages during the isolation of Enkephalinase A and B.

| Fraction | Tyr | | Tyr-Gly-Gly | | Tyr-Gly | |
|---|---|---|---|---|---|---|
| Homogenate | 7.2 | (61.5) | 3.4 | (2.2) | 30.5 | (3.9) |
| $S_1$ | 2.7 | (59.9) | 0.9 | (1.3) | 30.7 | (2.8) |
| $P_2$ | 5.5 | (30.8) | 6.9 | (5.7) | 4.1 | (2.5) |
| $S_2$ | 0 | (56.9) | 0 | (2.2) | 5.7 | (2.7) |
| $P_2$ | 2.1 | (27.3) | 7.2 | (5.6) | 4.2 | (1.8) |
| $S_3$ | 0 | (40.1) | 0 | (0.4) | 2.1 | (0.9) |
| $P_3$ | 1.0 | (27.2) | 9.0 | (7.3) | 2.5 | (3.1) |
| $R_3^*$ | 4.4 | (55.8) | 7.6 | (3.3) | 12.3 | (3.7) |
| $S_4^*$ | 0.3 | (57.3) | 2.7 | (2.0) | 5.8 | (4.6) |
| $R_4^*$ | 1.8 | (47.6) | 6.7 | (4.7) | 2.9 | (5.0) |
| $R_4^{**}$ | 3.2 | (33.9) | 7.1 | (5.7) | 6.2 | (7.4) |
| $S_5^{**}$ | 1.4 | (46.3) | 6.6 | (3.9) | 4.0 | (4.5) |
| $P_5^{**}$ | 0 | (24.4) | 2.1 | (2.0) | 1.0 | (1.6) |
| Enk. A | 0.4 | (2.6) | 25.8 | (28.5) | 0.9 | (2.3) |
| Enk. B | 0.3 | (1.9) | 1.1 | (1.6) | 34.8 | (35.2) |

Values in parentheses correspond to product formation in absence of puromycin. Other conditions as described in Fig. 1.

It is difficult to quantitate the amounts of aminopeptidase and Enkephalinase B in the washed particles because their activity is masked: ultra-sonification, room-temperature ageing or treatment with detergent cause a 2-5 fold increase in the activity of these enzymes (cf. Fig. 1). The most likely explanation of the above observations is that the soluble enzymes are occlused within the precipitate. Support to this idea is the observation that incubation with low concentrations of Triton X 100 (0.05-0.1 %) liberates the majority of the aminopeptidase and Enkephalinase B activity. Such low concentrations of detergent are not effective in detaching particle-bound enzymes but are capable in disorganizing membraneous structures. The enzymes released by such treatments (ageing, sonification or detergent) are soluble since they are not precipitable by ultracentrifugation (280 000 x g, 2h).

The bulk of the Enkephalinase A activity remains attached to the particles and we therefore used particles treated with 0.1% Triton X 100 as starting material for our Enkephalinase A preparation.

As shown in Fig. 1 and Table I, large amounts of Enkephalinase B are present in the 17 000 x g supernatants of striatal homogenates. Attempts to isolate this enzyme from such supernatants failed because it was not possible to remove the huge amounts of aminopeptidase also present. Better results have been obtained using the 50 000 x g supernatants, obtained after incubation of the particles with 0.1% Triton X 100.

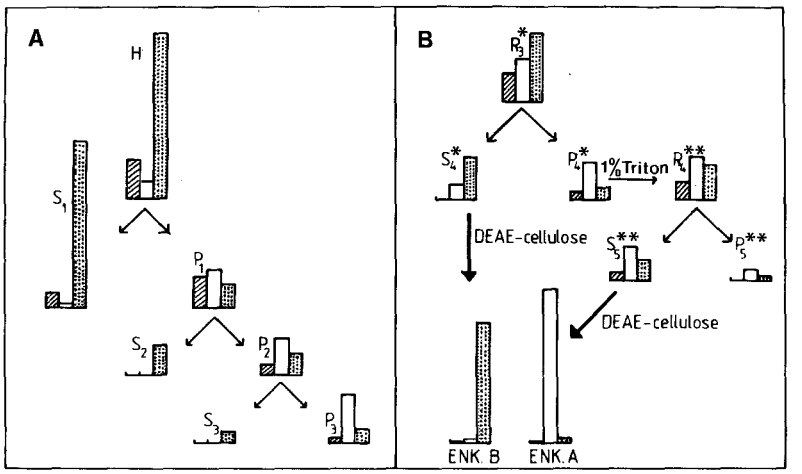


**Fig. 1.** Isolation of Enkephalinase A and B from calf-brain striatum.

The bars in the histograms represent the formation of Tyr (▨); Tyr-Gly-Gly (□) and Tyr-Gly (⊡), in 20 min at 37°C, expressed as % of the amount of substrate originally present.

Abbreviations, H = 1 : 6 homogenate (w/v) of striatum in Tris. HCl buffer pH 7.5; P = precipitate and S = supernatant obtained after centrifugation at 17 000 x g (Fig. 1A) and 50 000 x g (Fig. 1B) respectively. $R^*_3$ is obtained after resuspension of $P_3$ in 0,1% Triton X 100 and extraction for 1 h at room temperature, and $R^{**}_4$ is obtained similarly, except that the Triton X 100 concentration is 1%. Enzymatic activity was measured as described in Methods. For reasons of comparison the supernatants and precipitates have been deluted to give the same volume as the original homogenate.

The final Enkephalinase A and B preparations, purified 30 respectively 20 fold with respect to the crude homogenate are not completely free of contaminating enkephalin-degrading enzymes, but since more than 95% of the Leu-enkephalin hydrolysis in the Enkephalinase A preparation is due to splitting of the $Gly^3$-$Phe^4$ bond and about 94% of the activity in the Enkephalinase B to splitting of the $Gly^2$-$Gly^3$ bond, it is safe to use these preparations to investigate the properties of the respective enzymes.

Angiotensin-converting enzyme activity is virtually absent in the Enkephalinase A preparation since omission of captopril (an ACE inhibitor) during the incubation does not alter the amount of Tyr-Gly-Gly produced by this preparation.

2.Kinetic parameters.

The solubilization and/or subsequent purification procedures do not affect the kinetic parameters of neither Enkephalinase A nor B. This is concluded from the data in Fig. 2 where Lineweaver-Burk plots for the hydrolysis of Leu-enkephalin by crude and purified enzymes are shown. The $K_m$ values calculated by linear regression are 30 µM for Enkephalinase A and 10 µM for Enkephalinase B, respectively. Fig. 3a shows the effect of the pH on the activity of the two enzymes. Enkephalinase B has a broad maximum between 7 and 9 while Enkephalinase A has a much sharper optimum situated somewhat below 7. We also determined the effect of the pH on

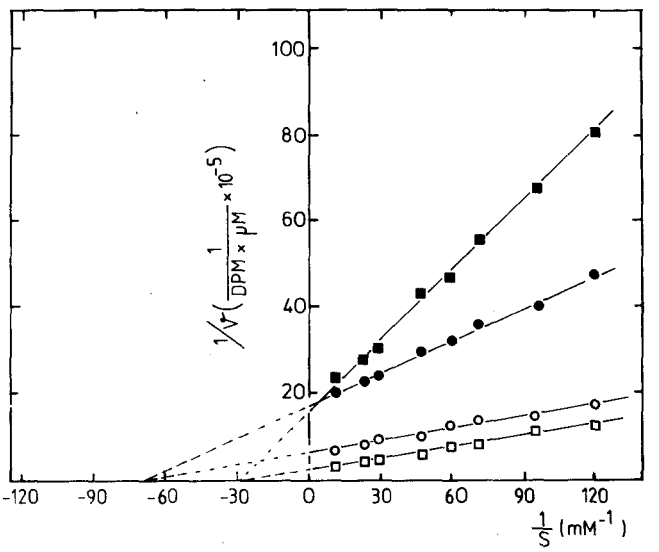


**Fig. 2.** Lineweaver-Burk plots for calf-brain enkephalinases in crude and purified preparations.

The rate of Leu-enkephalin degradation was measured with 8 concentrations of of enkephalin (100 - 10 µM) with increasing specific activity (6.7-67 mCi/mmole). Incubation, 20 min at 37°C. Other conditions as described in Methods and Materials. ■---■, Enkephalinase A, membraneous preparation; □---□, Enkephalinase A, purified enzyme; ●---●, Enkephalinase B, membraneous preparation and O---O, Enkephalinase B, final preparation.

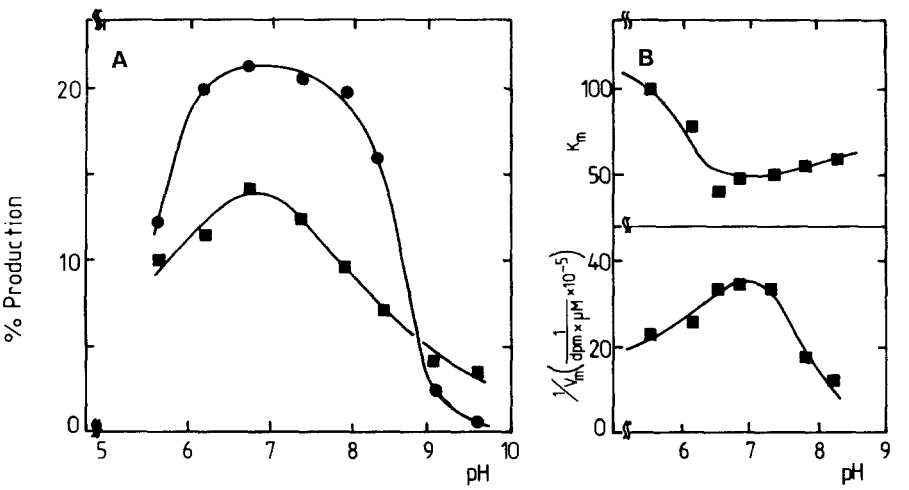


**Fig. 3.** Effect of the pH on the activity of purified Enkephalinase A and B.

**A.** pH profile for the hydrolysis of 10 µM Leu-enkephalin. The amounts of product formed in 20 min are expressed as percentage of the initial amount of Leu-enkephalin. The buffer used was a mixture of equal concentrations (50 mM) of HEPES buffer, cacodylic acid and glycine, adjusted to the desired pH with NaOH. ■---■, Enkephalinase A and ●---●, Enkephalinase B.

**B.** pH profiles for the $K_m$ and $V_m$ of Enkephalinase A. The kinetic parameters were determined from Lineweaver-Burk plots. HEPES buffer, adjusted to the desired pH values with NaOH was used in all experiments. Conditions as described in Fig. 2.

the $K_m$ and V of Enkephalinase A (Fig. 3b). The turnovernumber has a minimum value around pH = 7 and depends somewhat more on the pH than the $K_m$ but in the pH range measured (5.5-8.5), there are only small effects of the pH on the affinity and the catalytic-centre activity of Enkephalinase A.

Rat-brain Enkephalinase A is inhibited rather effectively by phosphate (4,9,12). Calf-brain enzyme is also inhibited by phosphate as shown in Fig. 4. Phosphate is a purely competitive inhibitor and from the Dixon-plot (see Inset) a $K_i$ of 1.5 mM was determined (pH = 6.85). The $K_i$ depends on the pH: at pH 7.4 a $K_i$ of 6.5 mM was found.

Rat-brain Enkephalinase A is strongly inhibited by Thiorphan (13) and for reasons of comparison we also measured the effects of Thiorphan on calf-brain Enkephalinase A and B as (Fig. 5). It is a purely competitive inhibitor for Enkephalinase A with a $K_i$ of 3 nM (inset Fig. 5). We also measured the inhibition of calf-brain enkephalinases by secobarbital and found that Enkephalinase A is about as sensitive to this drug as the rat-brain enzyme (see Table II and Ref. 14).

Enkephalinase B is not inhibited by any of the Enkephalinase A inhibitors mentioned above (Table II). This enzyme is, however, (like Enkephalinase A) sensitive for metal chelators like EDTA and o-phenantroline.

According to Gorenstein and Snyder (4) simple dipeptides containing tyrosine or phenylalanine inhibit the enkephalin hydrolysis. Depending on the type of dipeptide either Enkephalinase A, or B or both are inhibited. As the inhibitory effects of

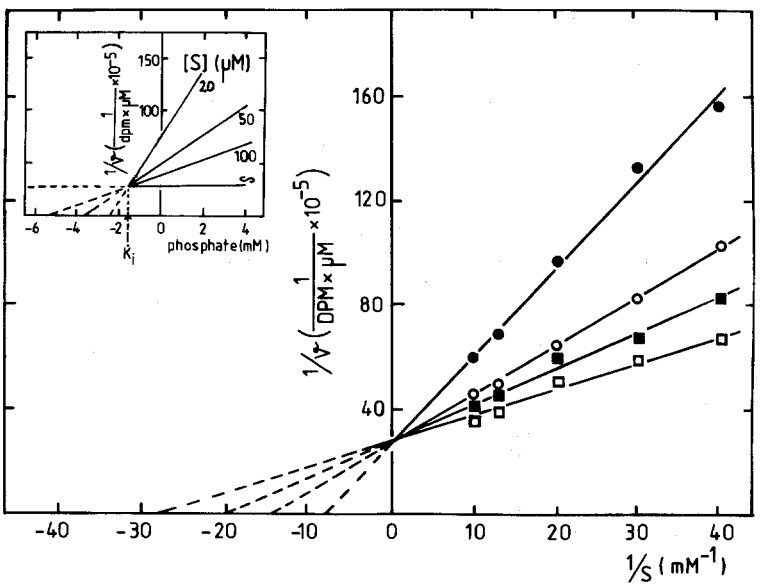


**Fig. 4.** Lineweaver-Burk plots for Enkephalinase A in the presence of phosphate. The activities were measured essentially as described in Fig. 2 except that varying concentrations of phosphate were present. pH = 6.85 in all experiments. □---□ , no phosphate; ■---■ , with 0.5 mM phosphate; O---O, with 1 mM phosphate and ●---●, with 3 mM phosphate.
Inset: Dixon plot for the inhibition of Enkephalinase A by phosphate.

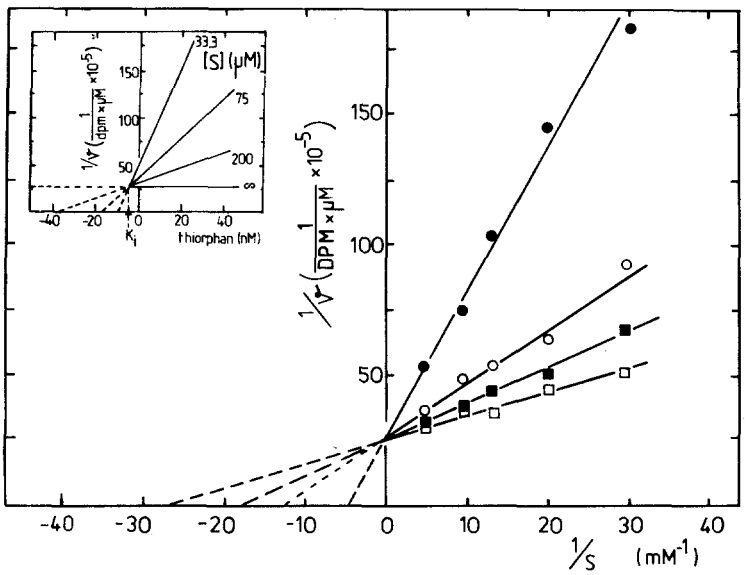


**Fig. 5.** Effect of Thiorphan on the activity of calf-brain Enkephalinase A.

□---□ , without Thiorphan; ■---■ , in the presence of 3 nM Thiorphan; O---O, in the presence of 10 nM Thiorphan and ●---●, in the presence of 30 nM Thiorphan. Ohter conditions as in Fig. 2.
Inset: Dixon plot for the inhibition of Enkephalinase A by Thiorphan.

such molecules can be used for rational design of enkephalinase inhibitors we have extended the measurements of Gorenstein and Snyder. The results, summarized in Table III, show that dipeptides with an extra positive charge (Tyr-Arg and Tyr-Lys) are recognized by both enzymes. Enkephalinase B has a slightly higher affinity than Enkephalinase A for these dipeptides. Neutral dipeptides also react with both enzymes but the differences in affinity are larger. The largest difference is observed

**TABLE II**

Inhibitors of purified calf-brain Enkephalinase A and B.

| | | % of control | |
|---|---|---|---|
| Inhibitor | Concentration | Enkephalinase A | Enkephalinase B |
| none* | - | 100 | 100 |
| Puromycin | 0.1 mM | 96 | 99 |
| Puromycin | 1.0 mM | 105 | 103 |
| Bacitracin | 0.1 mM | 98 | 99 |
| Captopril | 1.0 μM | 101 | 98 |
| Phosphate | 50 mM | 2 | 102 |
| Secobarbital | 0.1 mM | 35 | 104 |
| Thiorphan | 0.1 μM | 1 | 98 |
| EDTA | 0.1 $mM^+$ | 95 | 78 |
| EDTA | 1.0 $mM^+$ | 23 | 14 |
| o-Phenantroline | 0.1 $mM^+$ | 58 | 64 |
| o-Phenantroline | 1.0 $mM^+$ | 3 | 2 |

*, Activity was measured in absence of captopril and puromycin.
+, Enzyme preincubated with inhibitor at 37°C for 10 min.

TABLE III
Inhibitory potency of various dipeptides on purified calf-brain Enkephalinase A and B.

| | % of control | | | % of control | |
|---|---|---|---|---|---|
| Dipeptide* | Enk. A | Enk. B | Dipeptide* | Enk. A. | Enk. B |
| Tyr-Lys | 76 | 36 | Phe-Tyr | 37 | 8 |
| Tyr-Arg | 70 | 45 | Leu-Tyr | 73 | 12 |
| Tyr-Gly | 83 | 80 | Gly-Tyr | 100 | 90 |
| Tyr-Ala | 42 | 17 | Gly-Phe | 100 | 86 |
| Tyr-Val | 87 | 9 | Leu-Gly | 100 | 78 |
| Tyr-Leu | 81 | 16 | Arg-Gly | 75 | 80 |
| Tyr-Tyr | 63 | 6 | | | |
| Tyr-Phe | 51 | 4 | Tyr-D-Arg | 99 | 101 |
| Tyr-Glu | 100 | 14 | Gly-D-Phe | 100 | 89 |

*,Concentration = $10^{-4}$ M.

when the terminal aminoacid has an extra negative charge: Tyr-Glu does not react with Enkephalinase A but it effectively inhibits the Enkephalinase B activity.

## DISCUSSION

With our sensitive HPLC techniques we could measure the activities of Enkephalinase A, B and aminopeptidase simultaneously during all stages of the isolations. Some remarkable results emerge from such studies (Table I, Fig. 1).

Enkephalinase B is a soluble enzyme and its presence in membraneous preparations is solely due to occlusion. We have shown, that simple washing of the precipitates with buffer is not effective in removing all Enkephalinase B activity from the particles and also that, although latent in fresh preparations, the Enkephalinase B activity can increase during the incubations simply by ageing at elevated temperatures (25-37°C) or by the presence of small amounts of surface-active agents (detergents and/or certain drugs). It is this uncontrolled increase in activity that may lead to misinterpretation of results of experiments directed to elucidate the structure and function of membrane-bound Enkephalinase A when such crude preparations as $P_2$ in Fig. 1 are used. Of course when both Tyr-Gly and Tyr-Gly-Gly are measured simultaneously one can correct for the competing activity of Enkephalinase B. But the fact, that the affinity of Enkephalinase B is 2-3 times larger than that of Enkephalinase A and the unpredictable rate of the demasking of Enkephalinase B activity in particulate preparations, remain serious complicating fators.

The aim of this study was to obtain Enkephalinase A and B preparations with the same properties as the native enzymes and we did not try to obtain chromatographically pure preparations: the often drastic procedures needed to purify membrane-bound enzymes to that extent can easily introduce artifacts. Our preparations have the same activity for their natural substrates as they have in their membrane-bound (or occlused) state and we are convinced that their properties reflect as close as possible the in-vivo situation. The properties of Enkephalinase A and B from calf-brain striatum are very similar to those reported for the rat- and mouse-brain enzymes (9,11-15) but because of the easy avaliability of large quantities of enzyme,

we prefere the calf-brain enzymes in studies of the structure-activity relations of enkephalinases .

The $K_i$ for inorganic phosphate increases with increasing pH. This is explained by assuming that the $H_2PO_4^-$ ion has the highest affinity for the active site of Enkephalinase A. This conclusion in in line with the findings of Vogel and Altstein (15) who observed a shift in the pH optimum of rat-brain Enkephalinase A upon increasing the phosphate concentration in their incubation medium.

Though binding the same substrate with comparable effectivity ($K_m$ = 1-3.$10^{-5}$M), the active sites of Enkephalinase A and B differ considerably in their ability to recognize certain inhibitors: phosphate and Thiorphan are several orders of magnitude more effective in inhibiting Enkephalinase A than Enkephalinase B. This difference could be explained if Enkephalinase B does not have a Zinc atom in its active site. This is, however, not likely in view of the large sensitivity of this enzyme to metal chelators (Table II and Ref. 4.). As we do not yet know much about the spatial array of the active site of Enkephalinase B, an explanation for the difference between the two enzymes would be highly speculative and should wait until more detailed information becomes available.

The potency of small inhibitors can be used to obtain more insight in the structure of the active site. Table III shows, that the lateral amino acids are most important for the binding of the substrate to the enzyme: dipeptides containing two large side chains are effective inhibitors while dipeptides with the small glycin as terminal amino acid are hardly effective as inhibitors. The most interesting observation is the difference in potency between L-Tyr-L-Arg and L-Tyr-D-Arg. These dipeptides, named "Kyotorphins" because of their discovery in the Kyoto University, are reported to elicit anti-nociceptiviness when administered intra-cisternally in rats (16). Though the L isomer inhibits both Enkephalinase A and B this inhibition can not be correlated with the analgesic effects of this compound: the D-Arg isomer is not at all effective in the in-vitro tests while in the in-vivo tests its potency is much larger than the L-isomer. The results on the inhibition by dipeptides show again the difference between the two enzymes: while Tyr-Tyr and Tyr-Phe inhibit Enkephalinase B effectively they have a low affinity for Enkephalinase A. This is in agreement with an earlier report of Gorenstein and Snyder (4) for the rat-brain enkephalinases. Less agreement exists between the sensitivity of calf-brain Enkephalinase A and mouse-brain Enkephalinase A for Tyr-Gly: the mouse enzyme is severely inhibited by $10^{-5}$ M Tyr-Gly (Refs 4,14,17) while the calf enzyme is hardly affected by $10^{-4}$ M of this dipeptide. Low affinity for Tyr-Gly is also found by Sullivan et al. (18): they reported that in the presence of $10^{-3}$ M Tyr-Gly rat-brain Enkephalinase A still had 15% of its original activity. The reason for such species-dependent differences in sensitivity for inhibitors is not clear and further investigation is needed before observations on rodent enzyme systems can be extrapolated to that of humans.

REFERENCES

1. Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, A., Morgan, B.A. and Morris, H.R. (1975) Nature 258, 577-579.
2. Dupont, A., Cusan, L., Garon, M., Alvarando-Urbina, G. and Labrie, F. (1977) Life Sci. 21, 907-916.
3. Craves, F.B., Law, P.Y., Hunt, C.A. and Loh, H.H. (1978) J. Pharmacol. Exp. Ther. 206, 492-506.
4. Gorenstein, C. and Snyder, S.H. (1980) Proc. Roy. Soc. Lond. B 210, 123-132.
5. Schwartz, J.-C., Malroy, B., and de la Baume, S. (1981) Life Sci. 29, 1715-1740.
6. Hazato, T., Inagaki-Shimamura, M., Katayama, T. and Yamamoto, T. (1982) Biochim. Biophys. Res. Commun. 105, 470-475.
7. Van Amsterdam, J.G.C., Van Buuren, K.J.H., Dik, J. and Wynne, H.J. (1983) J. Chrom. 258, 165-174.
8. Mahy, N., Tusell, J.M. and Gelpi, E. (1982) J. Chrom. 233, 115-122.
9. Gorenstein, C. and Snyder, S.H. (1979) Life Sci. 25, 2065-2070.
10. Van Amsterdam, J.G.C., Van Buuren, K.J.H., Wynne, H.J. and Nolet, M.R.A. (1982) Pharm. Weekblad (Sci. Ed.) 4, 106.
11. Vogel, Z. and Altstein, M. (1977) FEBS Lett. 80, 332-336.
12. Vogel, Z. and Altstein, M. (1980) Prog. Biochem. Pharmacol. 16, 49-59.
13. Roques, B.P., Fournié-Zaluski, M.-C., Soroca, E., Lecomte, J.M., Malfroy, B., Llorenz, C. and Schwartz, J.-M. (1980) Nature 288, 286-288.
14. Vogel, Z. and Altstein, M. (1979) in Way, E.L., Ed., "Endogenous and Exogenous Opiate Agonists and Antagonists", pp. 353-356, Pergamon Press, New York.
15. Llorens, C., Gacel, G., Swerts, J.-P., Perdisot, P., Fournié-Zaluski, M.-C., Schwartz, J.-C. and Roques, B. (1980) Biochim. Biophys. Res. Commun. 96, 1710-1716.
16. Takagi, H., Shiomi, H., Ueda, H. and Amano, H. (1979) Eur. J. Pharmacol. 55, 109-111.
17. Hudgin, R.L., Charleson, S.E., Zimmerman, M., Mumford, R. and Wood, P.L. (1981) Life Sci. 29, 2593-2601.
18. Sullivan, L., Alkil, K., Blecker, D. and Barchas, J.D. (1980) Peptides 1, 31-35.