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# Purification of Carboxypeptidase B from Human Pancreas

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Carboxypeptidase B of the human pancreas was purified by chromatography on DEAE-cellulose and CM-cellulose columns. Two forms of the enzyme, named carboxypeptidase $B_1$ and $B_2$, were separated. They have similar mol.wts. ($34250 \pm 590$) as established by polyacrylamide-gel disc electrophoresis and by gel filtration. Carboxypeptidase $B_2$ migrates further towards the anode in disc electrophoresis. When the amino acid content of the enzymes was analysed, carboxypeptidase $B_2$ had four more glycine and three more aspartic acid residues than had form $B_1$. The amino acid sequence of the human carboxypeptidase $B_1$ differs from that of the bovine enzyme only in two places in the *N*-terminal 20-amino-acid sequence. The *N*-terminal amino acid in carboxypeptidases $B_1$ and $B_2$ is alanine. The peptide 'map' of the tryptic digest of carboxypeptidase $B_1$ contained more peptides than did that of form $B_2$. The $K_m$, the $V_{max.}$ and the pH optimum of the cleavage of the peptide substrate hippurylarginine and the ester substrate hippurylargininic acid were similar for both enzymes. $CoCl_2$ accelerated the peptidase activity, and cadmium acetate enhanced the esterase activity, of human carboxypeptidases $B_1$ and $B_2$. Urea and sodium dodecyl sulphate inhibited the enzymes.

Although pancreatic carboxypeptidases have been studied extensively (Neurath *et al.*, 1970; Folk *et al.*, 1960), most of the available information deals with enzyme from non-human sources. Because studies (Kemmler *et al.*, 1971) on the conversion of proinsulin into insulin by pancreatic carboxypeptidase B (EC 3.4.12.3) underline the physiological importance of this enzyme, we decided to purify the human pancreatic enzyme and study some of its properties. In the course of this investigation we isolated two proteins, named carboxypeptidases $B_1$ and $B_2$, which differ in the number of negative charges that they carry.

## Experimental

### *Materials*

Normal tissues were obtained from the Department of Forensic Pathology of this Center. The tissues were washed with 0.9% NaCl at 4°C and were stored frozen at −80°C until used. The substrates used in the assays were obtained from the following sources: hippurylargininic acid (benzoylglycine α-hydroxy-δ-guanidino-*n*-valeric acid ester) from Cyclo Chemical Co., Los Angeles, CA, U.S.A.; hippuryllysine (benzoylglycyl-lysine) and hippurylarginine (benzoylglycylarginine) from Calbiochem, La Jolla, CA, U.S.A.; DEAE-cellulose, type 70, from Sigma Chemical Co., St. Louis, MO, U.S.A.; CM-cellulose 32 from Whatman (W. and R. Balston, Maidstone, Kent, U.K.); Sephadex G-100 and Blue Dextran 2000 from Pharmacia Fine Chemicals, Uppsala, Sweden. Dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride was obtained from Sigma. Standard proteins were obtained as follows: bovine serum albumin, ovalbumin, human haemoglobin and lysozyme from Sigma; pepsin and α-chymotrypsin from Worthington Biochemical Corp., Freehold, NJ, U.S.A. The reagents for polyacrylamide-gel electrophoresis were purchased from Canalco, Rockville, MD, U.S.A.

### *Enzyme preparation*

Human pancreases obtained from autopsy were washed with cold 0.9% NaCl and rapidly frozen.

The preparation of an acetone-dried powder, precipitation with $(NH_4)_2SO_4$ and chromatography on DEAE-cellulose were done as described by Folk *et al.* (1960), except that in the chromatography the Tris/HCl buffer was 0.05 M and the NaCl gradient was from 0 to 0.5 M (Fig. 1).

### *Chromatography on CM-cellulose*

Carboxypeptidases $B_1$ and $B_2$ were separately chromatographed on Whatman CM-cellulose 32 in 0.01 M-potassium phosphate buffer, pH 6.7, with a linear gradient ranging from 0.01 (pH 6.7) to 0.05 M-potassium phosphate buffer, pH 8.5 (see Fig. 2 below).

*Protein determination*

During isolation and purification procedures the protein concentrations were determined by measuring the $A_{280}$ of the solution or by the method of Lowry *et al.* (1951), with bovine albumin as standard.

*Enzyme assay*

The hydrolysis of the ester and peptide substrates was measured in a Cary model 15 or 118 C recording u.v. spectrophotometer at 254nm, as described by Folk *et al.* (1960). The substrates used were hippuryl-argininic acid, hippuryl-lysine and hippurylarginine at 1mM in a 0.1M-Tris/HCl buffer, pH7.4. If not otherwise indicated, the temperature was kept at 37°C. The effect of pH on the rate of hydrolysis of substrate was studied in a wide-range buffer containing 0.05M-sodium citrate, -sodium phosphate (dibasic) and -sodium borate.

In the inhibition studies, the inhibitors were preincubated with the enzymes for 20min at 22°C. The $K_m$ and $V_{max.}$ were calculated by plotting $1/v$ against $1/s$ (Lineweaver & Burk, 1934).

*Amino acid analysis*

Carboxypeptidases $B_1$ and $B_2$ were hydrolysed in 5.7M-HCl in vacuum-sealed tubes at 105°C for 24, 48 and 72h. The data for serine and threonine were extrapolated to zero time. Cysteine and cystine were determined by the performic acid-oxidation method with the previously described corrections (Hirs, 1967). Tryptophan was determined spectrophotometrically (Goodwin & Morton, 1946; Beaven & Holiday, 1952). In each hydrolysis experiment, 1mg of freeze-dried enzyme protein was used. The amino acid content was measured with a Beckman 121 automatic amino acid analyser.

*Disc electrophoresis*

Both purified carboxypeptidases were subjected to polyacrylamide-gel disc electrophoresis at pH8.9. About 50$\mu$g of protein was applied to each gel, and gels were stained with Coomassie Brilliant Blue R-250 and destained with 7% (v/v) acetic acid (Davis, 1964).

*Determination of molecular weight*

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was done by the method of Shapiro *et al.* (1967) as an additional test of purity and for determining the molecular weight. Protein (100$\mu$g) was incubated for 3h at 37°C with 1% sodium dodecyl sulphate and 1% 2-mercaptoethanol. Electrophoresis was carried out for 3h at 6mA per tube in 5% (w/v) polyacrylamide containing 0.05M-sodium phosphate buffer, pH7.2, and 0.1% sodium dodecyl sulphate. The gels were calibrated with standards of the following mol.wts.: bovine serum albumin (68000), ovalbumin (45000), pepsin (35000) and $\alpha$- and $\beta$-chains of human haemoglobin (16000). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of proteins was generally carried out in quadruplicate. The relative mobility, $R_m$, of each protein (maximum = 1) could be measured with a precision of 2%, which would introduce an error of about 4% in the estimation of molecular weight. Least-squares analysis of the data fitted the equation:

$$\log M = 5.4966 - 1.5516\ R_m$$

where $M$ denotes the molecular weight of the protein or polypeptide. The plot of $\log M$ versus $R_m$ was linear over the mol.wt. range 16000–130000 and had no inflexion.

*Determination of molecular weight by gel filtration*

A column (100cm×2.5cm diam.) of Sephadex G-100 was equilibrated and eluted with 0.05M-Tris/HCl buffer, pH7.4; fractions (4ml/tube) were collected. The flow rate was 30ml/h. The column was calibrated with standards of the following mol.wts.: bovine serum albumin (68000), ovalbumin (45000), $\alpha$-chymotrypsin (23800) and lysozyme (14400). The results of the gel filtration of marker proteins fit the following equation obtained by the method of least squares:

$$\log M = 5.5550 - 0.5949(V_e/V_0).$$

*Peptide 'mapping' and sequencing*

Enzyme (1mg of protein) was dissolved in 0.1ml of 0.2M-*N*-ethylmorpholine/acetic acid buffer, pH8.5, and 10$\mu$l of a 0.5% solution of trypsin in water and one drop of toluene were added. The mixture was incubated for 12h at 37°C and subsequently freeze-dried. The freeze-dried peptides were dissolved in 0.1ml of 0.1M-$NaHCO_3$ and treated with the dansyl chloride reagent (Gray & Hartley, 1963). The dried samples were dissolved in acetone/acetic acid (3:2, v/v), then subjected to t.l.c. on polyamide sheets (Woods & Wang, 1967). The fluorescent peptides were localized under u.v. light.

The *N*-terminal amino acid of the $B_1$ and $B_2$ enzymes and the sequence of the *N*-terminal 20 amino acids in carboxypeptidase $B_1$ were determined on a Beckman sequencer (Mole *et al.*, 1975) by Dr. F. D. Capra of this Center.

## Results

*Purification*

During the purification procedures described above, two B-type carboxypeptidases were separated.

Table 1. *Purification of carboxypeptidases $B_1$ and $B_2$ from human pancreas*
A unit of carboxypeptidase activity is defined as 1 $\mu$mol of hippurylargininic acid cleaved/min.

| Purification step | Volume (ml) | Total activity (units) | Protein (mg/ml) | Sp. activity (units/mg of protein) | Yield (%) | Purification (fold) |
|---|---|---|---|---|---|---|
| Extraction | 1590 | 98660 | 5.6 | 11 | 100 | 1 |
| $(NH_4)_2SO_4$ precipitation (60% satn.) | 420 | 77083 | 10.6 | 18 | 78 | 1.6 |
| DEAE-cellulose column chromatography | | | | | | |
| Form $B_1$ | 106 | 35298 | 0.8 | 441 | 36 | 40 |
| Form $B_2$ | 210 | 32665 | 2.9 | 54 | 33 | 4.9 |
| CM-cellulose column chromatography | | | | | | |
| Form $B_1$ | 86 | 20504 | 0.2 | 1192 | 21 | 108 |
| Form $B_2$ | 42 | 14488 | 0.4 | 862 | 15 | 78 |

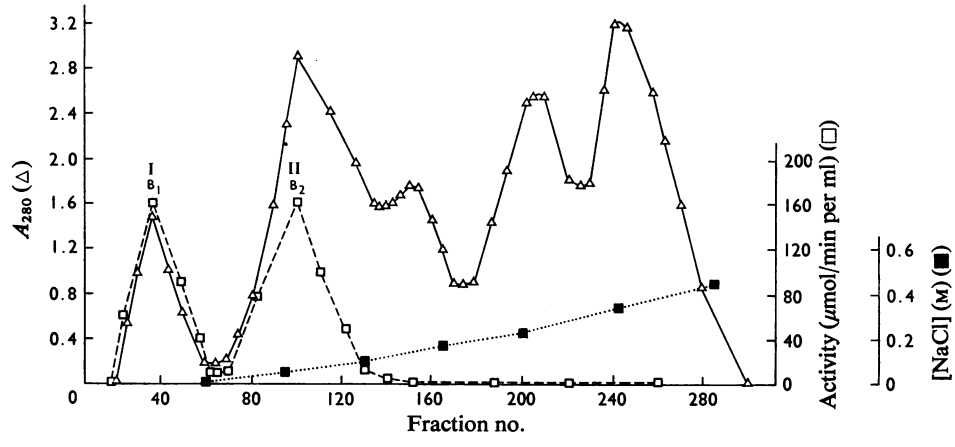


Fig. 1. *DEAE-cellulose column chromatography of human pancreatic carboxypeptidase B obtained by precipitation by 60%-satd. $(NH_4)_2SO_4$*
For details, see the text. A dialysed sample (200ml) was applied to the column (75cm × 2.5cm diam.). Fractions (8.5ml/tube) were collected, and the flow rate was 60ml/h. The enzyme activity appeared in two peaks, $B_1$ and $B_2$. The activity was eluted with 0.005M-Tris/HCl buffer, pH7.5, containing NaCl increasing in concentration from 0 to 0.5M (■). △, $A_{280}$ (protein); □, enzyme activity. Hippurylargininic acid was the substrate in Figs. 1, 2*a* and 2*b*.

Table 1 summarizes the purification steps used. After extraction of carboxypeptidase B from human pancreas, precipitation with $(NH_4)_2SO_4$ increased the purification relatively little. DEAE-cellulose column chromatography, however, resolved the activity into two separate peaks, carboxypeptidases $B_1$ and $B_2$ (Fig. 1). The first sharp peak of enzyme activity (form $B_1$) appeared in the fraction eluted before the salt gradient was applied. Fractions with carboxypeptidase activity (form $B_2$) were eluted when the NaCl concentration in the eluent increased from 0.01 to 0.1M. The recovery of carboxypeptidase activity after DEAE-cellulose chromatography was 88%, 46% in peak $B_1$ and 42% in peak $B_2$. The material in each peak, $B_1$ and $B_2$, was purified separately on a CM-cellulose column. Carboxypeptidase $B_1$ was eluted from this column with 0.022M-phosphate buffer, pH7.25 (Fig. 2*a*). Carboxypeptidase $B_2$ was similarly eluted with 0.016M-phosphate buffer, pH7.2 (Fig. 2*b*). The second, smaller, peak of activity was caused by carboxypeptidase $B_1$, since in disc electrophoresis (see below) this protein migrated the same distance as form $B_1$.

Both carboxypeptidase $B_1$ and $B_2$ hydrolysed the ester and the two peptide substrates tested. Measuring the activity in the peaks eluted from the CM-cellulose column with the peptide substrate hippurylarginine instead of the ester substrate hippurylargininic acid

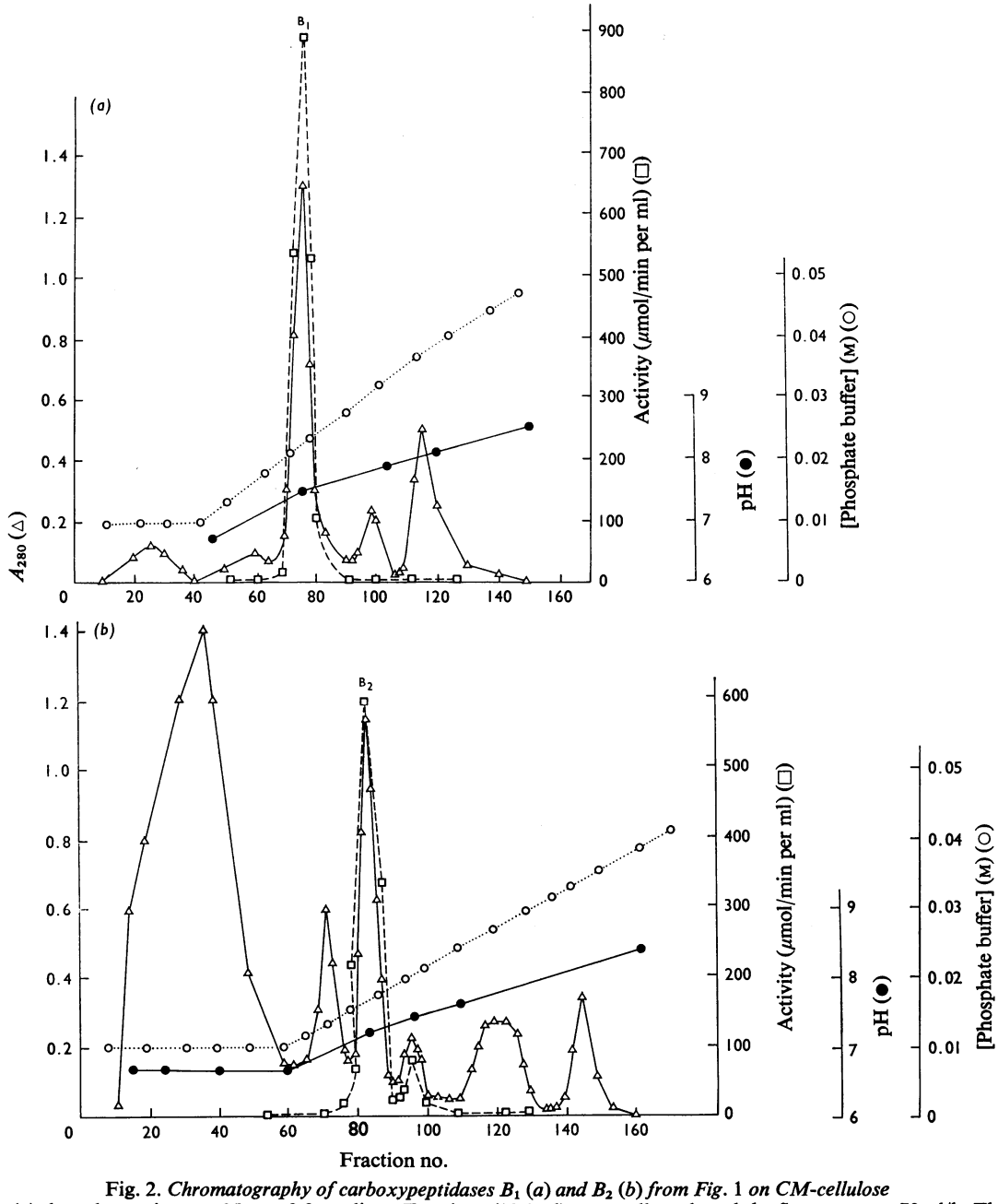


Fig. 2. *Chromatography of carboxypeptidases $B_1$ (a) and $B_2$ (b) from Fig. 1 on CM-cellulose*
In (*a*) the column size was 35cm×2.2cm diam. Fractions (10.3ml) were collected, and the flow rate was 70ml/h. The enzyme was eluted with potassium phosphate buffer increasing linearly in molarity (○) and pH (●). For further details, see the text. △, $A_{280}$ (protein); □, activity. In (*b*) the column size was 32cm×2.2cm diam. Fractions (8.6ml) were collected, and the flow rate was 60ml/h. The conditions and symbols were otherwise as described in (*a*).

yielded symmetrical peaks, identical with the ones shown in Figs. 2(*a*) and 2(*b*).

In common with pig carboxypeptidase B (Folk *et al.*, 1960; Folk & Gladner, 1961), human carboxypeptidases $B_1$ and $B_2$ cleaved the ester bond in hippurylargininic acid faster than the amide bond in hippurylarginine. At the substrate concentration used in the spectrophotometer, namely 1mM,

hippurylargininic acid was hydrolysed by the crude homogenate 3.8 times more rapidly than was hippuryl-arginine. Both carboxypeptidase $B_1$ and $B_2$ cleaved hippurylarginine faster than hippuryl-lysine (2.1 and 2.0 times at 1 mM). Table 2 shows the $V_{max.}$ of the hydrolysis of hippurylargininic acid was higher, and the $K_m$ lower, than the values for hippurylarginine.

*Electrophoresis*

Both carboxypeptidase $B_1$ and $B_2$ yielded a single band in disc electrophoresis, but form $B_2$ migrated faster toward the anode. (In addition, a faint faster-migrating band was observed in the form-$B_2$ preparation, possibly due to a trace impurity.) The rate of migration of forms $B_1$ and $B_2$ in 5% (w/v) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was 0.63 relative to that of haemoglobin (1.0).

*Molecular weight*

The differences in rate of migration disappeared in the presence of sodium dodecyl sulphate, indicating that the molecular weights of carboxypeptidases $B_1$ and $B_2$ are similar, although they carry different charges, $B_2$ being more acidic at pH 8.9. The mol.wt. of carboxypeptidases $B_1$ and $B_2$ was estimated to be 34250 (±590) by disc electrophoresis. Values about 5% lower were obtained when the molecular weight was established by gel filtration on a column of Sephadex G-100: that of form $B_1$ was found to be 32920 (±600) and that of $B_2$, 32210 (±450).

*Amino acid analysis*

Carboxypeptidase $B_1$ contains an estimated minimum of 302 amino acid residues per molecule and the calculated number of residues in form $B_2$ is 316 (Table 3). The biggest difference between the two isoenzymes was that form $B_2$ contained three more aspartic acid and four more glycine residues.

*Sequencing and digestion*

Alanine is the *N*-terminal amino acid in both carboxypeptidase $B_1$ and $B_2$. The sequence of the

Table 2. *$K_m$ and $V_{max.}$ values of carboxypeptidase $B_1$ and $B_2$*

| Enzyme | Substrate | $K_m$ (mM) | $V_{max.}$ (μmol/min per mg) |
|---|---|---|---|
| Carboxypeptidase $B_1$ | Hippurylargininic acid | 0.050 | 1560 |
| | Hippurylarginine | 0.277 | 555 |
| Carboxypeptidase $B_2$ | Hippurylargininic acid | 0.071 | 1250 |
| | Hippurylarginine | 0.310 | 336 |

Table 3. *Amino acid composition of carboxypeptidases $B_1$ and $B_2$ from human pancreas*

The number of residues per molecule is the average (±S.E.M.) of three analyses after 24, 48 and 72 h of hydrolysis, based on a mol.wt. of 34500, except for the threonine and serine values, which were extrapolated to zero time of hydrolysis. Methionine was determined as methionine sulphone.

| | No. of residues per molecule | | Nearest integer | |
|---|---|---|---|---|
| Amino acid | $B_1$ | $B_2$ | $B_1$ | $B_2$ |
| Lysine | 17.67 (±0.80) | 17.03 (±0.64) | 18 | 17 |
| Histidine | 5.88 (±0.34) | 6.40 (±0.16) | 6 | 6 |
| Arginine | 14.62 (±0.62) | 15.05 (±1.20) | 15 | 15 |
| Aspartic acid | 21.95 (±1.06) | 25.20 (±2.03) | 22* | 25* |
| Threonine | 24.7 | 25.6 | 25 | 26 |
| Serine | 22.8 | 21.8 | 23 | 22 |
| Glutamic acid | 30.96 (±1.19) | 33.50 (±2.77) | 31 | 33 |
| Proline | 12.56 (±0.53) | 13.96 (±1.64) | 13 | 14 |
| Glycine | 24.40 (±0.97) | 28.10 (±1.18) | 24* | 28* |
| Alanine | 31.58 (±1.23) | 31.76 (±2.34) | 32 | 32 |
| Half-cystine | 3.80 (±0.05) | 3.70 (±0.10) | 4 | 4 |
| Valine | 10.88 (±0.41) | 12.98 (±0.79) | 11 | 13 |
| Methionine | 1.76 (±0.03) | 2.23 (±0.23) | 2 | 2 |
| Isoleucine | 17.96 (±0.54) | 18.91 (±1.53) | 18 | 19 |
| Leucine | 22.18 (±0.68) | 23.10 (±1.88) | 22 | 23 |
| Tyrosine | 17.25 (±0.91) | 17.65 (±1.75) | 17 | 18 |
| Phenylalanine | 12.29 (±0.56) | 12.41 (±0.99) | 12 | 12 |
| Tryptophan | 7 | 7 | 7 | 7 |
| Total no. of residues | | | 302* | 316* |
| Mol.wt. | | | 33560 | 34960 |

* Significantly different ($P < 0.05$).

first 20 *N*-terminal amino acids in human carboxypeptidase $B_1$ is different only in two positions from that of the bovine enzyme. Bovine carboxypeptidase B has an *N*-terminal threonine (Titani *et al.*, 1975) and glutamic acid in position 20. In contrast, the human enzyme has glutamine in position 20. The sequence is: Ala-Thr-Gly-His-Ser-Tyr-Glu-Lys-Tyr-Asn-Asn-Trp-Glu-Thr-Ile-Glu-Ala-Trp-Thr-Gln.

*Peptide 'map'*

Peptide 'maps' of the tryptic digest of the carboxypeptidases $B_1$ and $B_2$ are clearly different (Fig. 3). The $B_1$ enzyme yielded three peptides not present in form $B_2$, whereas $B_2$ yielded a single additional peptide spot. The other peptides show a similar pattern of migration.

*Effects of pH*

The effect of pH on the hydrolysis of hippurylargininic acid by carboxypeptidase $B_1$ or $B_2$ was similar. The pH optimum of the esterase action of both enzymes was above 10. Carboxypeptidase $B_1$ and $B_2$ cleaved the peptide substrate hippurylarginine fastest at pH7. Carboxypeptidase $B_1$, however, had a broader pH optimum because the activity of form $B_2$ decreased at a steeper rate at pH values above 8 (Fig. 4).

*Inhibition and activation*

Carboxypeptidases $B_1$ and $B_2$ were activated and inhibited similarly by various agents (Table 4). The

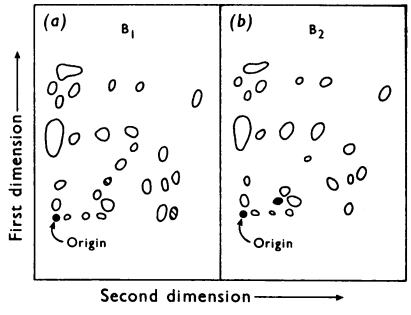


Fig. 3. *Separation of products of tryptic digestion of carboxypeptidases (a) $B_1$ and (b) $B_2$ in two-dimensional t.l.c.*
The solvent in the first dimension was water/90% (v/v) formic acid (200:3, v/v) and in the second dimension benzene/acetic acid (9:1, v/v). The positions of the peptides were located under u.v. light and tracings of the spots are shown. The dark and the shaded spots show the differences between the tryptic digests of forms $B_1$ and $B_2$.

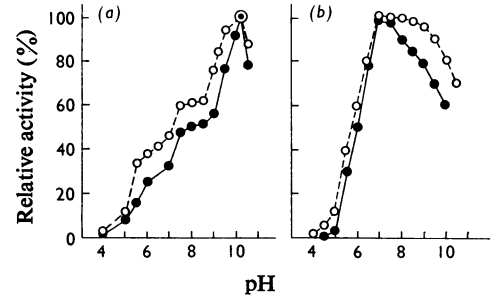


Fig. 4. *Effect of pH on the rate of hydrolysis of hippurylargininic acid (a) and hippurylarginine (b) by carboxypeptidase $B_1$ (○) and $B_2$ (●)*
For details, see the text. Activity is expressed as a percentage of the maximum rate. The concentration of the substrates was 1 mM.

Table 4. *Effects of inhibitors and activators on human pancreatic carboxypeptidases $B_1$ and $B_2$*
The enzymes were dialysed against water and assayed for enzymic activity as described in the Experimental section. The enzymes were incubated with the activator or inhibitor for 20min at 20°C before the assay. Results are expressed as a percentage of the activity in the absence of added materials.

| | | Relative activity (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | Substrate ... | Hippurylargininic acid | | Hippurylarginine | | Hippuryl-lysine | |
| Inhibitor or activator | Concn. | $B_1$ | $B_2$ | $B_1$ | $B_2$ | $B_1$ | $B_2$ |
| EDTA | 3.3mM | 82 | 79 | 85 | 69 | 100 | 82 |
| Cadmium acetate | 0.5mM | 150 | 178 | 48 | 42 | 31 | 24 |
| $CoCl_2$ | 1mM | 90 | 80 | 124 | 170 | 138 | 161 |
| Sodium dodecyl sulphate | 0.01% | 0 | 0 | 0 | 0 | 0 | 0 |
| Urea | 2M | 53 | 61 | 36 | 50 | 19 | 20 |
| | 4M | 33 | 48 | 25 | 33 | 5 | 10 |
| | 8M | 0 | 0 | 0 | 0 | 0 | 0 |
| ε-Amino-*n*-hexanoic acid | 3mM | 72 | 71 | 63 | 60 | 38 | 18 |

hydrolysis of the three substrates by human pancreatic carboxypeptidases $B_1$ and $B_2$ was only partially (0–31%) inhibited by 3.3mM-EDTA. As observed with the pig enzyme (Folk *et al.*, 1960; Folk & Gladner, 1961), $CoCl_2$ accelerated the peptidase activity when measured with hippurylarginine and hippuryl-lysine substrates, but partially inhibited the esterase action, i.e. the hydrolysis of hippuryl-argininic acid. Cadmium acetate, however, accelerated the esterase but inhibited the peptidase activity. Both forms $B_1$ and $B_2$ were sensitive to sodium dodecyl sulphate and urea: 0.01% sodium dodecyl sulphate completely inhibited the enzymic activity, and 4M-urea inhibited carboxypeptidase $B_1$ and $B_2$ 52–95%. The esterase activity, however, was inhibited less by 2M-urea than was the peptidase action.

## Discussion

We report here the purification of two carboxypeptidase B-type enzymes from human pancreas. The difference between the two enzymes, carboxypeptidases $B_1$ and $B_2$, is that form $B_2$ seems to carry more negative charges. This conclusion was based on the differences observed during separation of forms $B_1$ and $B_2$ by ion-exchange chromatography, on the faster migration of form $B_2$ toward the anode in disc electrophoresis and on the analysis of the amino acid content. With the latter technique, after total hydrolysis, we found three more aspartic acid residues in form $B_2$ than in $B_1$. Peptide 'mapping' of the tryptic digest of the enzymes suggested that trypsin cleaves form $B_1$ in more places than $B_2$ (Fig. 3).

Others have observed several forms of pancreatic carboxypeptidase. Geokas *et al.* (1974) mentioned that human pancreatic juice collected from the duodenum has two carboxypeptidase B-type enzymes, but Clemente *et al.* (1972) found only one procarboxypeptidase B in the human pancreatic juice by using immunological techniques. Folk & Schirmer (1963) described three different forms of carboxypeptidase A (EC 3.4.12.2) in pig pancreas, and bovine pancreatic juice has three different forms of carboxypeptidase A (Reeck *et al.*, 1971). Marinkovic & Marinkovic (1975) separated two different forms of carboxypeptidase A from human pancreas, and purified and characterized one of them.

After the completion of our experiments, we noticed a report by Geokas *et al.* (1975) describing the purification of carboxypeptidase B from human pancreatic juice. The enzyme had a mol.wt. of 23500 and an additional 9200-mol.wt. component. Our preliminary studies indicate that the 34000-mol.wt. form of human pancreatic carboxypeptidase B may be broken down by proteolytic cleavage to a protein of mol.wt. 24000. The possibility exists that a similar process *in vivo* yields an active but lower-molecular-weight product of the pancreatic enzyme.

Although the *N*-terminal amino acid of the human enzyme is different from that of the bovine one, the sequence of the next 18 amino acids is identical in human carboxypeptidase $B_1$ and in bovine carboxypeptidase B (Titani *et al.*, 1975). The bovine enzyme, however, has a glutamic acid residue at position 20, whereas the human enzyme has a glutamine residue there.

Pig and human pancreatic carboxypeptidase B also have many properties in common. The total numbers of calculated residues in the human pancreatic carboxypeptidases $B_1$ and $B_2$ are 302 and 316 respectively, which are close to the 304 residues reported for pig carboxypeptidase B (Folk *et al.*, 1960). $CoCl_2$ accelerates the hydrolysis of the peptide substrates but inhibits the cleavage of the ester substrate both by human and by pig (Folk & Gladner, 1961) carboxypeptidase B. Cadmium acetate acts in the opposite manner; it accelerates ester cleavage and inhibits peptide hydrolysis. EDTA inhibited carboxypeptidases $B_1$ and $B_2$ only partially. In contrast, human and pig plasma carboxypeptidase (carboxypeptidase N or arginine carboxypeptidase, EC 3.4.12.7) are completely inhibited by EDTA, and cadmium salts inhibit the hydrolysis of hippuryl-argininic acid (Erdös *et al.*, 1964, 1967; Jeanneret *et al.*, 1976) instead of accelerating it.

Presumably carboxypeptidase B acts mostly in the gastrointestinal tract. Only trace amounts of pancreatic carboxypeptidase B were found in human plasma (Geokas *et al.*, 1974), and most of the carboxypeptidase activity in blood is due to the presence of carboxypeptidase N (Oshima *et al.*, 1974, 1975). In addition to cleaving basic *C*-terminal amino acids from various proteins and peptides, pancreatic carboxypeptidase B may have an important function in converting proinsulin into insulin (Kemmler *et al.*, 1971). The presence of a carboxypeptidase B in the islets of Langerhans has further implicated the participation of this enzyme in insulin metabolism (Zühlke & Steiner, 1975). It remains to be seen whether the forms of the enzyme described in the present paper are involved in the conversion of proinsulin into insulin.

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