ON CHYMOTRYPSINOGEN AND TRYPSINOGEN BIOSYNTHESIS BY PANCREAS OF RATS FED ON A STARCH-RICH OR A CASEIN-RICH DIET

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Previous papers of this series (Reboud et al (1962) ; Ben Abdeljlil et al (1963, 1964))have shown that amylase is high and several proteolytic precursors (ChTg, Tg, ProCp B[1]) are low in pancreas and pancreatic juice of rats fed on a starch or glucose-rich diet (diet G). Conversely, amylase is low and proteolytic precursors are high when the animals receive a protein-rich diet (diet P). Amylase has been purified from pancreas of rats G and P after C^{14}-valine injection (Marchis-Mouren et al (1963)) and it has been found that diet G, when compared with diet P, induces a fourfold increase in the relative rate of biosynthesis of this enzyme. Similar experiments have now been performed with ChTg and Tg of rat pancreas.

10 pancreas G or P (about 7 g) are homogenized in 9 volumes of water, dilute H_2SO_4 being added to bring the final pH to 2.2. After centrifugation (50,000 g ; 45 min.), the clear supernatant is dialyzed against HCl 10^{-3}M and lyophilized. The powder is dissolved in H_2SO_4 M/200 and the solution (2 % protein) is preci-

1 The following abbreviations are used : ChTg, chymotrypsinogen; Tg, trypsinogen ; ProCp B, procarboxypeptidase B ; ATEE, acetyl-L-tyrosine ethylester ; BAEE, benzoyl-L-arginine ethylester . V_R, retention (or break - through) volume of the column.

pitated with ammonium sulfate. The precipitate between 0.15 and 0.40 saturation, containing about 80 % of the total ATEE and BAEE activity[2], is dialyzed overnight against HCl 10^{-3}M and then 2 x 2 h. against a 0.018 M acetate buffer at pH 4.50. Figure 1 gives the results of a chromatography of this solution on sulfo-ethyl-cellulose.

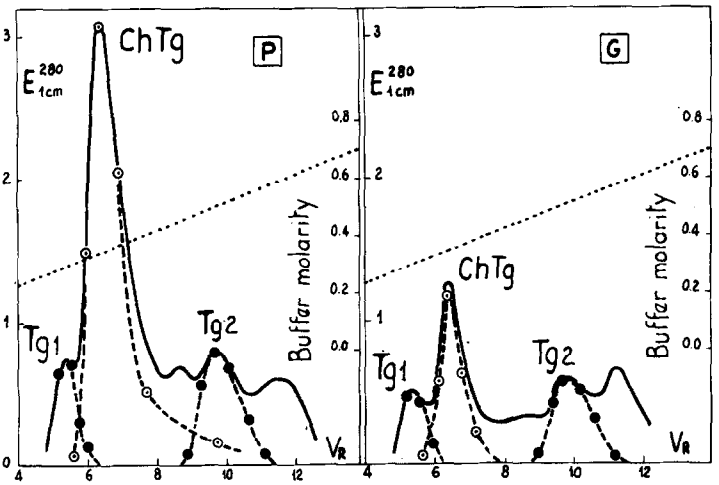

Figure 1. Purification of rat ChTg and Tgs on sulfo-ethyl-cellulose at pH 4.50. The acid extracts (see text) are obtained from homogenates of pancreas P or G containing exactly the same amount of DNA. Column (0.9 x 18 cm ; V_R, 8 ml) equilibrated with a 0.018 M acetate buffer pH 4.50 and eluted (0.5 V_R/h. ; fractions of 0.17 V_R (1.3 ml)) by a linear gradient of the buffer concentration (Elution yield, 70-75 % for the 3 precursors. Solid line, total proteins. Interrupted line, activity of the fractions against ATEE (white circles) or BAEE (black circles). The ATEE and BAEE activities have been ajusted in such a way that their maximal values coincide in diagram P with the maximal protein values. Dotted straight line, concentration gradient of the buffer.

In figure 1, one ATEE-positive and two BAEE-positive peaks are seen. The ATEE-positive peak corresponds to the anionic ChTg already identified in rat pancreatic juice by DEAE-cellulose

2 Activation and determination of activity against ATEE and BAEE have already been described (Ben Abdeljlil et al,1964).

chromatography at pH 8.0 (Marchis-Mouren et al, 1963). Rechromatography of its central part under the same conditions gives a single peak with constant specific activity[3]. Rechromatography of the central part of the second BAEE-positive peak emerging at 10 V_R also gives a single peak at the same position with no detectable BAEE activity in the 5-6 V_R region. Therefore, the first BAEE positive peak is not likely to be an artefact and rat pancreas probably contains two anionic Tgs (ratio,1 :3), Tg_1 and Tg_2.

Diagrams P and G of figure 1 also show that about 3 times as much ChTg and about twice as much Tg is obtained from pancreas P than from an equivalent amount (on a DNA basis) of pancreas G. Thus, the actual amounts of proteolytic enzymes, not merely their activities, are higher in pancreas P.

On the other hand, C^{14}-valine incorporation into ChTg, Tg_1, Tg_2 and total proteins by rats P and G is given in Table I. In all assays, 4 µmoles of DL-C^{14}-valine (5 mC/mmole) are injected intraperitoneally into 10 rats P or G. After 10 or 15 min., the 10 pancreas are homogenized together. ChTg and the two Tgs are purified from an aliquot and total proteins are obtained from the remainder by the technique of Schneider. Radioactivity

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3 The maximal specific activity of rat ChTg is 425 when the proteins are estimated by the Lowry technique and 460 when the known absorbance ($E_{1\%}^{1\,cm}$ at 280 mµ = 18) of bovine ChTg A is used for the calculation. A similar evaluation ($E_{1\%}^{1\,cm}$ of bovine Tg = 14) gives 31 and 49 for Tg_1 and Tg_2, respectively. The value for rat ChTg is similar to the specific activity of pure bovine ChTg A (430) under the same conditions. The values for Tg_1 and Tg_2 are slightly lower than for bovine Tg (63).

(counts/min. corrected for self absorption) is determined in each sample and calculated for 1 mg P-DNA in the corresponding homogenate.

Table I

Radioactive valine incorporation into ChTg, Tg_1 Tg_2 and total proteins by pancreas P or G.

The radioactivity values should be multiplied by 10^3

Radioactivity incorporated into	Rats P		Rats G		Ratio P/G	
	10 min.	15 min.	10 min.	15 min.	10 min.	15 min.
Proteins (Pr)	304	700	516	555	-	-
ChTg	120	266	79.1	88.0	-	-
Tg_1	28.0	46.6	23.6	29.4	-	-
Tg_2	50.0	69.6	40.2	58.0	-	-
ChTg/Pr	0.39	0.38	0.15	0.16	2.6	2.4
Tg_1/Pr	0.092	0.067	0.046	0.053	2.0	1.3
Tg_2/Pr	0.160	0.100	0.078	0.100	2.0	1.0

The ratios of the radioactivity incorporated by rats P and G into the various proteins taken individually (P/G ratios) depend upon the respective evolution of the radioactivity in the valine pool from pancreas P and G. They are not significant as long as this evolution is unknown. On the contrary, the values ChTg/Pr, Tg_1/Pr and Tg_2/Pr are independant from the valine pool and, as in the case of amylase, their P/G ratios may be considered as indicating the influence of the diet on the relative rate of biosynthesis of the enzymes. It is seen that these ratios are 2.5 for ChTg and 1.0-2.0 for the Tgs. The lower value obtained

for the Tgs agrees well with our former evaluation of Tg levels in pancreas and pancreatic juice of rats P and G (Reboud et al (1962) ; Ben Abdeljlil et al (1963, 1964)). Thus, the amount of starch and protein in the diet appears to control, by a still unknown mechanism, the biosynthesis of amylase and proteolytic enzymes in rat pancreas.

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