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Extraction of Proteolytic Enzymes from Pancreatic Glands of Riyadh Slaughterhouse Animals

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Abstract. Pancreatic glands of different animals such as cattle, goat and sheep were used for the preparation of acetone dried powders of trypsin and chymotrypsin. The soluble fractions of enzymes were isolated from the minced pancreas with sodium chloride, precipitated with ammonium sulphate, dialysed and dried. The activities of the enzymes were determined using synthetic substrates, N-benzoyl-L-arginine-ethyl-ester hydrochloride for trypsin and N-acetyl-L-tyrosine ethyl ester for chymotrypsin. Maximum trypsin recovery in acetone dried poser obtained from 1 g of pancreatic tissue was found to be 135.1 units in ox. As regards the soluble fraction, cow pancreas produced maximum enzyme yield with a total activity of 106.28 units per/g of pancreatic tissue. Similarly maximum chymotrypsin recovery in acetone dried powder obtained from 1 g of pancreatic tissue was found to be 166.52 units in ox. As regards the soluble fractions cow pancreas yielded maximum enzyme activity of 93.38 units per g of pancreatic tissue.

Introduction

Slaughterhourse wastes are a very good source for the production of some enzymes such as proteolytic, lipolytic and amylolytic enzymes on laboratory, pilot and commercial scales. Trypsin and chymotrypsin are very important proteolytic enzymes in industry and medicine. Pancreatic glands have been proved to be a good source of a number of important proteins, such as insulin, trypsin, chymotrypsin, etc. [1]. It appears that quite a large number of animals are killed daily in Saudi Arabia and almost all the essential glands and organs which are not a normal part of the human diet go to waste. Therefore these glands and organs, particularly the pancreas which is a good source of a number of enzymes [2], can be obtained quite cheaply. The present work is therefore directed towards extraction and assay of trypsin and chymotrypsin from the pancreatic glands of various animals, such as ox, cow, goat and sheep.

Materials and Methods

Preparation of pancreatin

Pancreatic glands of different animals were collected immediately after killing and preserved in an ice-salt chilling mixture for transfer to the laboratory for processing.

The chilled pancreas was then cut into small pieces. These pieces were frozen in liquid nitrogen and ground to a fine powder, using a pestle and mortar.

Ground pancreating glands were blended 3 times with 4 volumes of acetone for fifteen minutes on each occasion using a top drive homogenizer. The bucket of the blending machine was kept in an ice-salt mixture during blending. After each treatment, the suspension was recovered by centrifugation. Then the residue was treated with acetone: diethyl ether (1:1) mixture and then with diethyl ether. The filter cake thus obtained was dried to a constant weight in a desiccator to give a fine powder known as pancreatin. It was preserved in a bottle and stored in a desiccator over phosphorus pentoxide. The percentage of pancreatin obtained from the pancreatic glands of different animals is given in Table 1.

Isolation of proteolytic enzymes

Minced pancreatic glands were stirred in 10% (w/v) NaCl solution (six times the wt. of pancreatic glands) using a magnetic stirrer for about half an hour. The suspension was filtered through a nylon net and the filtrate (t) was preserved. The filter cake was stirred again with 10% sodium chloride solution (four times the wt. of filter cake) for 15 minutes. The suspension was filtered again through the nylon net and similarly a third extract of the pancreas was collected. All the 10% NaCl extracts were combined. The combined 10% NaCl extract pancreas was precipitated with ammonium sulphate, at 75% saturation in the final mixture. The whole filtrate was dialysed

Table 1. Percentage yield of pancreatin obtained from pancreatic glands of different animals.

Animal	Weight of the ground pancreas (g)	Weight of pancreatin powder (g)	Percentage of * pancreatin powder
Cow	45.00	8.93	19.84
Ox	83.28	13.09	15.72
Sheep	25.54	3.90	15.27
Goat	27.40	4.69	17.12

* Average of triplicate.

against water at 5°C for 24 h to remove the ammonium sulphate and the precipitate formed was centrifuged, dried and named as "A".

To the filtrate (t) from which "A" was separated, acetone was added up to 80% saturation (100 ml filtrate + 400 ml acetone) to precipitate out the soluble protein. The precipitate was separated by centrifugation, dried and named as "B". The percentage yield of "A" and "B" is given in Table 2.

Activation of enzyme

The crude enzyme fraction Pancreatin and fraction "A" and "B" were activated separately by suspending them in 1% calcium chloride solution containing a small amount of crystalline trypsin (Sigma Chemical Co. Type XII-S) and kept in a refrigerator at 5°C.

25 mg of crude enzyme was suspended in 10 ml of 1% calcium chloride solution and 0.1 ml of 0.1% trypsin solution (0.1 unit of trypsin) (Sigma Chemical Co. Type XII-S) was added to it and the whole was placed in a refrigerator at 5° C for activation. After 24 h, the suspension, the solution, containing the activated enzymes i.e. trypsin and chymotrypsin, was collected.

Assay of trypsin

Trypsin was assayed according to the method described by Schwert and Takenaka [3]. N-benzoyl-L-arginine ethyl ester hydrochloride was hydrolysed under standard conditions at pH 9.5 and 30° C. The consumption of standard alkali during the reaction was recorded as a function of time. The manual titration procedure with a pH meter was used.

Table 2. Percentage yield of total enzymes obtained from pancreatic glands of different animals.

Animal	Weight of the ground pancreas (g)	Weight of "A"* (g)	Weight of "B"** (g)	Percentage yield of "A"	Percentage yield of "B"
Cow	45.00	2.41	1.79	5.36	3.98
Ox	146.47	2.01	2.63	1.37	1.80
Goat	26.66	0.31	0.38	1.16	1.43
Sheep	27.93	0.41	0.49	1.47	1.75

* The precipitates which were obtained after the precipitation of pancreatic extracts with ammonium sulphate were then dialysed and, centrifuged. These precipitates were named ("A").

** Precipitates were obtained from the filtrate left from "A" by addition of acetone were named as "B".

Reagents

i) 0.02M N-benzoyl-L-arginine ethyl ester hydrochloride:
Dissolve 0.69 g of N-benzoyl-L-arginine ethyl ester hydrochloride in 0.2 M borate buffer pH 9.5, and complete to 100 ml.
ii) 0.05 M sodium hydroxide solution.

Procedure

Ten ml of activated crude enzyme suspension, preadjusted to pH 9.5, was added to 10 ml of 0.02 M N-benzoyl-L-arginine ethyl ester hydrochloride and the mixture was stirred continuously. The temperature of the mixture was maintained at 30° C throughout the reaction and the pH was maintained at 9.5. The time at which the reaction was started was recorded. The pH was adjusted regularly to 9.5 by the addition of 0.05 M sodium hydroxide and the time and volume of alkali added were noted. The titration was carried out for up to 10 minutes.

One unit of trypsin was defined as the amount of enzyme which under the specific test conditions hydrolysed one micromole of N-benzoyl-L-arginine ethyl ester hydrochloride per minute.

Activities of all the samples, prepared as described before, were measured according to the procedure described above and are shown in Table 3.

Table 3. Activities of trypsin in the various samples prepared from the pancreas glands of different animals.

Sample	Activity in units	Specific activity in units/g	Total recovery per 1 g of pancreatic tissue
Ox pancreatin	21.5	860	135.1
Ox - A pancreatin	28.0	1120	15.34
Ox - B pancreatin	34.5	1380	24.78
Cow pancreatin	14.0	1240	66.41
Cow - A pancreatin	31.0	560	111.13
Cow - B pancreatin	27.0	1080	24.96
Sheep pancreatin	18.0	720	109.95
Sheep - A pancreatin	22.5	900	13.21
Sheep - B pancreatin	41.5	1660	29.12
Goat pancreatin	17.5	700	119.82
Goat - A pancreatin	20.5	820	9.53

Assay of chymotrypsin

N-acetyl-L-tyrosin ethyl ester was hydrolysed under standard conditions at pH 9.5 and 30° C. The volume of standard NaOH consumed during the reaction was recorded as a function of time. The manual titration procedure with a pH meter was used.

Reagents

i) 0.02M N-acetyl-L-tyrosine ethyl ester. Dissolve 0.50 g of N-acetyl-L-tyrosine ethyl ester in 0.2 M borate buffer pH 9.5 and complete to 100 ml.
ii) 0.05 M sodium hydroxide solution

Procedure

The procedure described under "Trypsin" was followed using 10 ml of 0.02 M N-acetyl-L-tyrosine ethyl ester instead of 10 ml of 0.02 M N-benzoyl L-arginine ethyl ester hydrochloride. The titration was carried out for up to 10 minutes and the corresponding values of time and alkali consumed were noted.

One unit of chymotrypsin was defined as that amount of enzyme which under the specified test conditions hydrolysed one micromole of N-acetyl-L-tyrosine ethyl ester per minute.

Activities of all the samples prepared as described before, were measured according to the procedure described above and are shown in Table 4.

Table 4. Activities of chymotrypsin in the various samples prepared from the pancreatic glands of different animals

Sample	Activity in units	Specific activity in units/g	Total recovery per 1 g of pancreatic tissue
Ox pancreatin	26.5	1060	166.61
Ox - A pancreatin	25.5	1020	14.00
Ox - B pancreatin*	30.0	1200	21.55
Cow pancreatin	19.5	780	154.79
Cow - A pancreatin	25.0	1000	53.56
Cow - B pancreatin	26.0	1040	41.37
Sheep pancreatin	20.5	820	125.22
Sheep - A Pancreatin	30.0	1200	17.62
Sheep - B pancreatin	23.0	920	16.14
Goat pancreatin	22.0	880	150.63
Goat - A pancreatin	24.5	980	11.40
Goat - B pancreatin	26.0	1040	18.25

Results and Discussion

As shown in Table 3, among the various dried acetone powders prepared from the pancreatic glands, that of ox gave maximum activity of trypsin, namely 860 units/g. This was followed by sheep, goat and cow, their activities being 720, 700 and 560 units/g, respectively. This shows that ox pancreas was the richest source of trypsin, although the difference in the activities of the samples from different animals was not great.

As regards the maximum total trypsin activity in the fractions obtained after extraction in saline water and precipitation with ammonium sulphate followed by dissolution and acetone precipitation, demonstrated that cow pancreas yielded the maximum enzyme with a total activity of 106.28 units per g of pancreatic tissue. This was followed by sheep, ox and goat which yielded total activities of 40.52, 39.76 and 22.96 units on the basis of 1 g of pancreatic tissue.

As shown in Table 4 among the dried acetone powders prepared from the pancreas of various species; that of ox gave maximum activity of chymotrypsin, namely 1060 units/g. This was followed by goat , sheep and cow, their activities being 880, 820 and 780 units/g, respectively. This shows that ox pancreas was the richest source of chymotrypsin, although the difference in the activities of the samples from different animals was not great.

As regards the maximum total chymotrypsin activity in the fractions obtained after extraction in saline and precipitation with ammonium sulphate followed by dissolution and acetone precipitation, cow pancreas yielded maximum enzyme with a total activity of 93.38 units per g of pancreatic tissue. This was followed by ox, goat and sheep which yielded total activities of 35.21, 33.83 and 33.47 units on the basis of 1 g of pancreatic tissue.

Conclusion

Although the extraction and precipitation procedure were uniform for all the pancreatic glands from different animals, it must be considered that a true comparison of the total activities is not possible. This is because, the enzymes of different origin might vary in their solubility and precipitation properties. However from the results presented in this study, the acetone dried powder from the ox pancreas showed the maximum trypsin and chymotrypsin activity, and among the solubel fractions, cow pancreas showed the maximum trypsin and chymotrypsin activity. However, all pancreas extracts studied yielded comparable amounts of enzyme.

References

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استخلاص بعض الإنزيمات الهاضمة للبروتينات من غدة البنكرياس في بعض الحيوانات المذبوحة بمسلخ الريـاض

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ملخص البحث. تهدف هذه الدراسة إلى التوصل إلى مصدر أو مصادر سهلة متوافرة واقتصادية لاستخلاص بعض الإنزيمات الهاضمة من غدة البنكرياس مثل التريسين (Trypsin) والكايموتريبسين (Chymotrypsin) والتي لها أهميتها في عالم الصناعة والطب.

وبما أن مسلخ الرياض هو من أكبر المسالخ التي يتم فيها ذبح أعداد من الثيران والأبقار والأغنام والضأن، فقد رؤى أن تتم دراسة المصدر من تلك الحيوانات.

جمع غدد البنكرياس ثم تمَّ تحضير الإنزيمات المشار إليها حسب طريقة (Henry) وعلى مستخلصين هما:

١) مستخلص الإنزيمات الجاف بالأسيتون.

٢) مستخلص الإنزيمات المذابة عن طريق الترسيب بمادة سلفات الأمونيوم.

تختبر فعالية الإنزيمات المفصولة على طريقة (Schwert and Takenaka) باستعمال أيضات صناعية مقابلة لتلك الإنزيمات.

أفضل المصادر هي:

أنزيم تريبسين: أكبر عائد لهذا الإنزيم وجد في استخلاص بنكرياس الثيران على الطريقة (١). . ففي كل واحد جرام يوجد حوالي (١٠, ١٣٥) وحدة فعّالة، وكان أكبر عائد من المستخلص على الطريقة (٢) من الأبقار. . ففي كل واحد جرام يوجد حوالي (٢٨, ١٠٦) وحدة فعّالة.

أنزيم كايموتريبسين: كانت النتيجة مماثلة للأولى تماما حيث أعطي مستخلص الطريقة (١) أكبر عائد من بنكرياس الثيران. . ففي كل واحد جرام حوالي (٥٢, ١٦٦) وحدة فعّالة، بينما مستخلص (٢) كان فيه العائد الأكبر من الإنزيم من بنكرياس الأبقار، ففي كل واحد جرام (٣٨, ٩٣) وحدة فعّالة.