Study of glucose-6-phosphate isomerase from pig skeletal muscle

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SUMMARY: A 4-step purification procedures were designed and proceeded for pig skeletal muscle glucose-phosphate-isomerase (GPI), an important enzyme in carbohydrate metabolism and admitted as a promising enzyme in study of meat quality. PAGE-pure GPI was obtained with following creteria:purification fold 10.86, specific activity 1.6×10^{5} units, PAGE spectrum showed one sole band, M.W.by SDS-PAGE method was 119,350, isoelectric point by IEF method was 6.5-6.6, optimum temperature 35°C, optimum pH 8, Michaelis-Menton constant 6.02mM, activation energy 7735.2 $cal/K^{-1}mol^{-1}$. The purified GPI had antigen property as identified by immunoelectrophoresis method with rabbit immune serum. The purification procedures are simple, easily manageable, the purity is high and the reproducibility is fairly good. Along with those property creteria, this study makes a good basis for further research in elucidating the relationships between enzymes and meat quality.

INTRODUCTION: In 1983, we studied the relationships between biochemical molecules and meat quality. We found that the activity of CPK of pig muscle was positively correlated with the content of creatine (ZENG,S.Y.1983) which was a characteristic of meat quality. Thereafter, we confirmed the reliability that CPK can be used as a biochemical index of meat quality by determining CPK activity using different methods. (ZENG,S.Y.1986).Based upon these results, we broaden the enzyme spectrum, detected the activities of some important enzymes in carbohydrate metabolism e.g.lactate dehydrogenase, glucose-6-phosphate dehydrogenase and GPI, etc. The results showed that the activity of GPI was positively correlated with that of CPK, and the activities of these two enzymes were both different in different anatomical locations of skeletal muscle. The activity values were consistent with the edible quality of different anatomical locations of muscles considering from the contents of water soluble proteins , hence the correlationship between GPI activity and meat quality was ascertained.(ZENG,S.Y.1987,1989). In order to make forword the research of the relationship between enzymes and meat quality, a difinite amount of enzyme preparation are needed. But owing to some practical problems, it is diffcult to obtain these enzyme preparation from our country or abroad. We attempted to isolate and purify them by ourselves. Firstly we prepared electrophoretic pure CPK from pig muscles, then we started to isolate and purify GPI. It was elucidated that the gene loci of GPI was inherently linked with that gene loci which may induce strain-sensitive in worse meat producing pigs.(MABRY,J.W.1981). Therefore if one would study this enzyme from determining its amino acid sequences to its gene level, it is necessary to obtain enough amount of this enzyme of a definite quality. Upon our experiences of isolation and purification of CPK (ZENG,S.Y. and LIO,Y.L.1990) and referred to the isolation and purification method in literatures of GPI from pea,(SUSUMU HIZUKURI et al 1976) rabbit muscle (NOLTMAN,E.A.1964), human cardiac muscle (ROBERT,W.G.et al 1976) etc., we designed a set of simple methods, isolated and purified GPI from 5 kinds of pig muscle and studied some of its properties. At the same time, we also isolated and purified GPI from 6 kinds of rabbit muscle and skeletal muscles from chicken and fishes as comparisons. The pathway was paved for the further research of the relationship between enzymes and meat quality.

MATERIALS: (1)Pig muscles Taken from Hangzhou Meat Factory directly after slaughter. Totally five kinds:M.longissimi dorsi M.psoas major, M.biceps femoris, M.quadriceps femoris, M.supraspinatus. And fresh pig muscles(back and hinder leg) bought from local salers slaughtered over night. (2)Rabbit muscles Rabbits for experimental use purchased from Zhejiang Medical Institute were bleeded to death. Six kinds of muscle were taken directly, five were the same as pig muscles, the sixth sample was M.lumbar eye. (3)Chick muscles Chicken bought from market, bleeded to death, two kinds of sample were taken: M.thorcis and biceps femoris. (4)Fish muscles Two kinds of fresh fish were bought from market, carp and silver carp. After killed, the muscles were taken.

INSTRUMENTS: Partition collector BJQ74 2 (Shanghai Medical Analytical Instruments Factory) Visible UV Spectrophotometer (UV 3000 Shimadzu and UV 7520 Shanghai The Third Analytical Instruments Factory) High Volt Electrophoresis instrument, Electrofo cusing instrument(both from Jiang Su Instrument Factory) Chromatographic columns (Shanghai Jing Hua Experimental Factory) REAGENTS: Sephadex G 100 (Shanghai Shen Hua Biochemical Technology Development Service) Low M.W.standard proteins, Glucose 6 phosphate(Shanghai Dong Feng BiochemicalReagents Factory) Other chemicals A.R.grade (Shanghai Reagent Shop)

METHODS: I)Isolation and purification of GPI The following procedures were designed and proceeded after consulting with literature reports and practicing repeatedly. 1)Crude extract Muscles were weighed and minced and extracted with 2 fold(in volume) quantity of pH 8.5, 50mM Tris solution in which 0.3g of EDTA and 0.7g of KCL were added. After 1hr. while with occas ional stirring, the fluid was filtered firstly with 4-layer cloth and then with filter paper. Thus the crude extract was ob tained. 2)Precipitation with methanol and chloroform The crude extract was precipitated with mixed solvents $CHCL_3$:CH_3OH (1:1), the quantity used was just that which made the color of the extract nearly disappeared. After stirring and setting, the supernatant was filtered. The filtered fluid was the solution after precipitation. 3)Dialysis The solution after pre cipitation was dialyzed against distilled water in refrigerator for 24hrs. The dialyzed solution was obtained. 4)Sephadex G-100 chromatography (WANG,D.S.1987) The gel was soaked in distilled water for more than 24hrs, and a column of 1.5/30cm was packed with distilled water. The elution begun with a buffer solution of pH8,30mM Tris containing 25mM NACL(about 50ml.) and followed by the same buffer solution containing 50mM NACL(about 150ml.) A partition collector was used to collect the eluate, 5ml/per tube.The enzyme activity and protein content were determined along with those of the step 1,2,3 solutions. II) Determination of enzyme activity 1)G-6-P method Referred to literature and modified 2)Activity unit definition The enzyme solution acts upon substrate (20mM G-6-P) at pH 8.5, 37 C. for 5 minutes, producing 1umol. F-6-P was difined as one unit. III) Protein determination (Manual 1981) 1)Biuret method Used for crude extract, solution after precipitation, dia lyzed solutions 2)Folin Denis method Used for solutions of all the four steps. 3)UV spectrographic method Used for column chromatography eluates. IV)Identification of purity: 1)Cellulose acetate electrophoresis To detect the electro-moving be havior of the purified enzyme solutions. 2)PAGE method (ZHANG,L.X.et al,1985) To test the purity of the purified enzyme so lutions. V)Properties of the enzyme 1) M.W. By SDS-PAGE method (ZHANG,L.X. and et al,1985), the M.W.of the subunit of the enzyme was determined. 2) Isoelectric point(pI) By electric focusing method (GUO,R.J.1988). 3)Amino acid analysis The purified enzyme solution after column chromatography were subjected to amino acid analysis with Hidachi 835-50 AA-Analyzer. 4) Kinetic properties The Km value, optimum pH and temperature and activation energy were determined by normal methods. 5) Antigenic property The rabbit immune serum was prepared (MENG,G.Z.1987) with purified enzyme solutions. By convection, micro immunoelectrophoresis etc (ZHANG,L.X.et al 1985), the antigenic property of the purified GPI solution was identified.

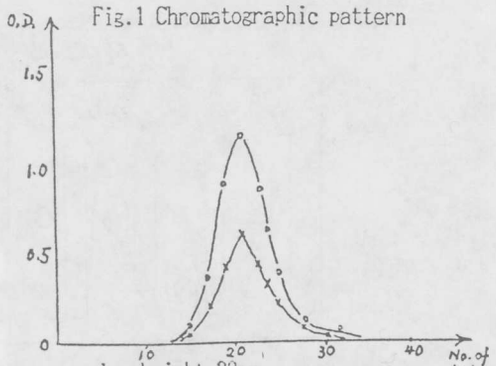
RESULTS

(I)Purification effect(Take M.biceps femoris as example)

Table 1

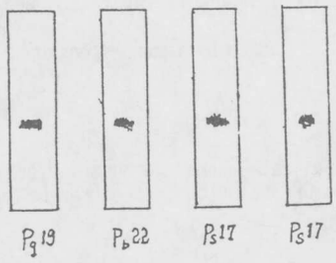
Item / step	total volume ml.	total protein mg.	total activity unitX10^4	activity recovery	specific activity unitX10^4	purification fold	No. of electro phoretic band
crude extract	67	1315.88	1996.60	100	1.51	1	>7
soln. after pption	60.3	425.71	1049.22	52.55	2.4	1.63	2
chromato graphic eluate	25.1	40.45	646.33	32.33	16.13	10.68	1

Fig.1 Chromatographic pattern



colum height 28cm
dialyzed solution 10ml.
protein A280nm
activity A490nm

Fig.2 Cellulose acetate electrophoretic pattern



P_g, P_b, P_g represent the 19th, 22th, 17th tube eluates of pig muscles M.quadriceps femoris, M.biceps femori M.supraspinatus respectively

(II)Purity identification See Fig.2, Fig.3

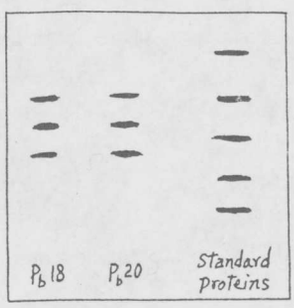
In Fig.2 all samples showed one spot only. The reproducibility was good. The three different muscle samples had the same electrophoretic behavior on cellulose acetate film.

III)Properties 1)M.W. From the SDS-PAGE spectrum and the calculation, it is known that the pig muscle GPI(from biceps femoris) is composed of three subunits. The M.W. of them determined from two separate samples purified in different time were fairly in agreement. They were: 53100,38050,28200; 52500,38300,28200. 2)Isoelectric point(pI) From Fig.5_1,5_2, it was estimated that the pI of GPI purified from pig muscle(M.biceps femoris) were 6.6, 6.5, that of rabbit muscle(as above) were 6.7 4.78, that of chicken muscle(as above) were 6.68 and 5.35. It was also clear from the spectra that electric focusing method can differentiate the components of the enzyme molecule more delicately than other electrophoretic methods, the details of the spectra of GPI from different muscles were different, but the main bands were the same.

Fig.3 PAGE spectrum of different steps of purification of GPI (pig muscle:M.quadriceps femoris)

C crude extract
P solution after precipitation
D dialyzed solution
E chromatographic eluate

Fig.4_1 SDS-PAGE spectrum



P_b18, P_b20 are the 18th, 20th tube eluates of pig muscle: M.biceps femoris

Fig.4_2 M.W.standard curve

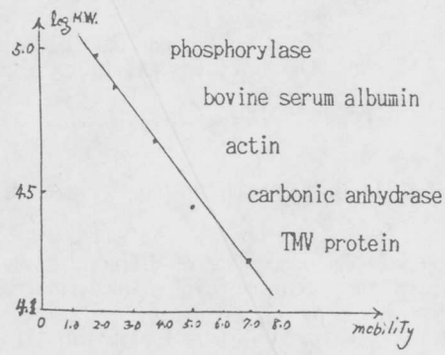
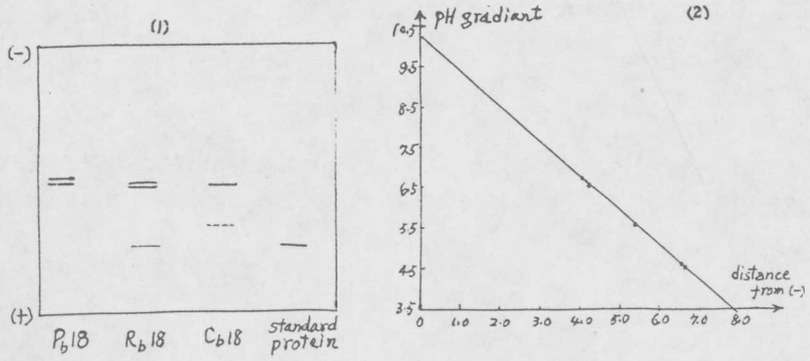


Fig.5 Iso electric focusing spectrum

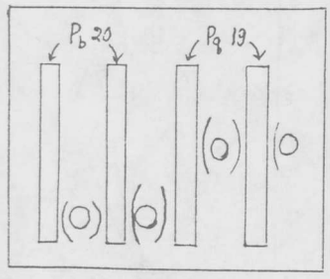


3) Amino acid analysis Data omitted here

4) Kinetic properties The Km value calculated from activity values at different substrate concentrations(5mM to 50mM G-6-P, eight concentrations)by linear regression method was 6.02mM. The activity energy as calculated from figure (omitted) of log activity versus different temperature(K°)and the Arrhenius equation: $\log\frac{a_1}{a_2}=\frac{E_2(T_2-T_1)}{2.303RT_1T_2}$, was 7735.2cal,($K^{-1}$,$mol^{-1}$)

5)Antigenic property The anti-serum prepared with the purified enzyme solution showed antigen antibody immune precipitation arc with the original enzyme solution as judged by immuno-electrophoretic spectra.

Fig.6 Immuno-electrophoretic spectra



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Because the antiserum was more complicated than the purified enzyme solution, we added anti serum in the hole and the purified GPI solution in the trough, just conversely as genera l procedures.

DISCUSSION AND CONCLUSIONS

This purification method is simple and concise. The whole procedures can be accomplished in four days, and can be processed in batch consectively. Purity determination can be advance after 2 or 3 runs of purification. The reproducibility of this me thod is fairly good(we puified this enzyme from pig skeletal muscles not less than 30 runs), the purity of the products is high, it can be used in scientific research works. We also obtained electrophoretic pure GPI from muscles of rabbit, chickens fishes etc. by this method, showing the applicability of this method is considerably wide, it can be used for muscles from various animals. Thus purified GPI added an item of enzyme preparation in enzyme industry of our country.

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