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Comparison of the Chromium Distribution in Organs and Subcellular Fractions of Normal and Diabetic Rats by Using Enriched Stable Isotope Cr-50 Tracer Technique

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ABSTRACT

The enriched stable isotope $^{50}Cr(III)$ tracer technique combined with neutron activation analysis was used to examine the intracellular distribution of Cr(III) in the liver, pancreas, testes, and kidney homogenates of both normal and diabetic rats. Our new results showed that the nucleic fraction has the highest Cr concentration in the liver cell of both normal and diabetic rats. The diabetic rats retain more Cr in the mitochondrial and lysosomal fractions of liver homogenate than the normal. This is likely an indication of chromium participating in the glucose or lipid metabolism to compensate the low level of insulin in the body of diabetic rats. The concentrations of Cr in the subcellular fractions of pancreas, testes, and kidney in the normal rats are higher than those in the diabetic rats, which favor the hypothesis that Cr(III) plays its biological function via interaction with the insulin-sensitive tissues or enhancement of the sensitivity of the insulin receptor.

Index Entries: Enriched stable isotope $^{50}Cr(III)$; subcellular distribution; diabetes; INAA.

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INTRODUCTION

Trivalent chromium is an essential trace element in human glucose and lipid metabolism. Its deficiency can cause diabeticlike symptoms *(1)*. Because diabetes is a series of endocrine abnormal metabolic disease, hormone-level and organic function changes might cause diabetic patients to be different at the absorption, storage, and excretion of dietary nutrients from healthy people. However, up to now, the function of chromium involved in carbohydrate and lipid metabolism is still unclear. Particularly, only a few studies are concerned with the behavior of Cr in the cell *(2)*, although a well-known fact is that the cell is the unit of structure of living things. Analysis of the intracellular distribution of Cr should shed some light on the formation of chromium-related biological macromolecules in cells and on its physiological and pathological roles. Additionally, the study of whether the different levels of insulin in animal bodies would affect the distribution of chromium in organs or intracellular compartments will also offer some important clues in understanding the relationship between chromium and insulin. Therefore, in this study, we examined the content of Cr in various tissues and their subcellular fractions of normal and diabetic rats, by using an enriched stable isotope Cr-50 compound as a tracer.

Generally, the Cr content in normal human and animal bodies is very low, only about tens of micrograms per kilogram. Thus, it is difficult to study the Cr distribution in subcellular fractions at such a low level. Furthermore, it is almost impossible to avoid the exogenous chromium contamination during the separation and determination processes. In addition, the radioactive tracer technique that was frequently used may cause radiation damage to cells, and the radioactive operation is not very convenient. Comparatively, using the enriched stable isotope Cr-50 tracer technique, one is able to avoid all the above shortcomings. The Cr concentration can be determined via $^{50}Cr(n, \gamma)^{51}Cr$ by neutron activation analysis, which is ideally suited for analyzing ultratrace elements in biological systems.

MATERIALS AND METHODS

Enriched Stable Isotope $^{50}Cr(III)$ Preparation

The enriched stable isotope Cr-50 compound $^{50}Cr_2O_3$ (the isotopic enrichment of Cr-50 is 94.2%) was purchased from the Institute of Atomic Energy (Beijing, China). The compound was dissolved by heating it in a few drops of concentrated perchloric acid, then a small amount of 2 mol/L hydrochloric acid and 30% hydrogen peroxide was added to reduce chromium to Cr(III).

Experimental Rats

Twelve male Wistar rats, weighing approximately 170±20 g, were obtained from the Center of Experimental Animals of Beijing Medical

University. Half of them were produced as the alloxan-induced diabetic rats. The detailed method was described in an earlier previous article *(3)*. The other half was used as controls.

The $^{50}Cr(III)$ tracer solution described was injected intravenously every 24 h at a dose of 50 μg Cr^{3+}/100 g body weight for 3 d. On the fourth day, all the rats were sacrificed and the organs were collected, washed with deionized water, and stored at –70°C until use.

Subcellular Fraction Separation

According to our previous metabolic experiments, the organs of the rat liver, kidney, pancreas, and testes with relatively high Cr contents were selected for the subcellular studies. These tissue samples were washed with a 10 m*M* HEPES/0.25*M* sucrose buffer (pH=7.5), then cut into strips by a Teflon knife, and homogenized with four to seven volumes of the buffer in a glass homogenizer. The homogenate was first centrifuged at 50*g* for 7 min to remove nondisrupted cells and then the supernatant was separated into different fractions by successive differential centrifugation *(4)*. It was done at 800*g* for 10 min to isolate nuclei, at 9000*g* for 10 min to isolate mitochondria, at 30,000*g* for 25 min to isolate lysosome, and at 100,000*g* for 2 h to isolate microsome in the residue and cytosol in the supernatant, by using a Beckman model 7L ultracentrifuge with a type 35 rotor. Each centrifugation step was done twice, and the residue was mixed together. All of the above operations were carried out at 4°C.

Chemicals and Determinations

All the reagents that were used were of the highest available purity. Deionized water was used throughout. Glassware was soaked in 1:1 nitric acid for over 1 wk and rinsed thoroughly with deionized water before use.

The protein concentrations in samples were assayed by the Coomassie Brilliant Blue G-250–protein binding method described by Bradford *(5)*.

The subcellular fraction samples were dried under an infrared lamp and covered by Teflon film and aluminum foil. All the samples and standards were irradiated in the Institute of Atomic Energy's heavy-water nuclear reactor at a flux of approx 6.0×10^{13} n/cm^2/s for 24 h. After 2 wk decay, the ^{51}Cr radioactivity was counted with a high-purity Ge detector with PC-based Ortec MCA.

RESULTS

Reference Materials and Blanks

A number of standard reference materials: NIST 1577a Bovine Liver, GBW 08551 Pork Liver, GBW 07602 Shrub Leaves, and GBW 07604 Poplar Leaves were analyzed for evaluating the accuracy of instrumental

Table 1
Cr Contents in Standard Reference Materials by INAA (μg/g)

SRM	This work	Certified value
Bovine Liver (NIST 1577a)	0.23 ± 0.07	0.2 ± 0.1 [6]
Pork Liver (GBW 08551)	0.16 ± 0.03	(0.2)* [7]
Shrub Leaves (GBW 07602)	0.55 ± 0.04	0.55 ± 0.05 [7]
Poplar Leaves (GBW 07604)	2.1 ± 0.1	2.3 ± 0.2 [7]

*Reference value. The number of determinations is six.

Table 2
Chromium Contents in Subcellular Fractions of Rat Liver

Subcellular	Cr Concentration μg/g		In Total Cell %		Cr/Total Protein [a] μg/g	
Fractions	N	D	N	D	N	D
Homogenate	0.42 ± 0.06	0.31 ± 0.09	—	—	1.96 ± 0.16	2.14 ± 0.16
Residue	0.59 ± 0.17	0.50 ± 0.20	21.5	11.7	1.57 ± 0.17	1.95 ± 0.20
Nuclei	2.01 ± 0.42	1.69 ± 0.26	27.0	33.3	5.14 ± 0.42	4.14 ± 0.26
Mitochondria	0.99 ± 0.23	1.39 ± 0.19	18.2	18.7	3.58 ± 0.23	5.87 ± 0.21
Lysosome	0.59 ± 0.17	0.55 ± 0.21	5.14	6.72	1.43 ± 0.18	2.87 ± 0.21
Microsome	0.11 ± 0.01	0.61 ± 0.15	0.883	10.9	0.29 ± 0.01	4.08 ± 0.15
Cytosol	0.13 ± 0.04	0.12 ± 0.05	27.3	18.8	1.98 ± 0.13	1.54 ± 0.05

[a] Cr/protein means the ratio of the amount of Cr to the total quantity of protein in the fraction. The meanings are the same in Tables 3–5.

Notes: Values are expressed as mean ± SD (N=6); N = normal and D = diabetic rats.

neutron activation analysis (INAA). The results are shown in Table 1. The agreement between our values and the certified values is quite good, confirming the good accuracy and precision of our procedures.

In our experiments, the blanks of the reagents and all the package materials for irradiation were analyzed by INAA, although the use of the enriched stable isotope tracer technique is ideal to minimize the contamination. The results indicated that no detectable Cr contamination in the whole procedure was found or the Cr content was negligible.

Chromium Contents in Subcellular Fractions of Rat Liver

The chromium contents in subcellular fractions of normal and diabetic rat liver are given in Table 2.

The liver homogenate of the normal rats has slightly higher Cr content than that of diabetic rats. In the subcellular distribution, the nuclei fraction contains the highest Cr values among all the fractions of both group rats. The Cr contents in various cell fractions of the normal rats

Table 3
Chromium Contents in Subcellular Fractions of Rat Pancreas

Subcellular Fractions	Cr Concentration μg/g		In Total Cell %		Cr/Total Protein μg/g	
	N	D	N	D	N	D
Homogenate	0.151±0.010	0.079±0.006	—	—	1.33±0.11	0.78±0.07
Residue	0.081±0.006	0.143±0.004	4.82	5.66	0.94±0.08	0.82±0.05
Nuclei	0.116±0.007	0.132±0.004	4.36	6.79	0.85±0.07	0.33±0.02
Mitochondria	0.252±0.010	0.174±0.009	7.24	6.79	1.24±0.08	0.25±0.02
Lysosome	0.308±0.008	0.161±0.009	3.50	4.98	1.08±0.06	0.54±0.04
Microsome	0.246±0.009	0.128±0.008	9.49	7.20	1.75±0.11	0.48±0.04
Cytosol	0.158±0.004	0.119±0.004	70.6	68.4	2.31±0.13	1.08±0.07

Notes: The pancreas of six rats were homogenized together and then separated into subcellular fractions. The chromium measurement error is less than 5%. N—normal rats; D—diabetic rats.

decrease in the order nuclei > mitochondria > lysosome > cytosol ≈ microsome, whereas for the diabetic rats, it decreases in the order nuclei > mitochondria > microsome ≈ lysosome > cytosol. Comparatively, Cr is mainly enriched in the nuclei, mitochondrial, and cytosol fractions. However, the Cr contents in the mitochondrial and microsomal fractions of the diabetic rats are significantly higher than those of the normal rats ($p < 0.001$). When expressed as Cr/protein, the Cr contents in the mitochondrial, lysosomal, and microsomal fractions of the diabetic rats is statistically higher than those of the normal rats ($p < 0.001$).

Chromium Contents in Subcellular Fractions of Rat Pancreas

Table 3 shows that the Cr content in the pancreatic homogenate of the normal rats is twice as that of the diabetic rats. In the intracellular distribution, the Cr contents in the fractions of the normal rats are statistically higher than those of the diabetic rats ($p < 0.05$), except in the nucleic fraction. The Cr content in nuclei is similar in both groups. In the relative percentage distribution, about 68–70% Cr is accumulated in the cytosol fraction. The Cr distribution pattern in the pancreas subcellular fractions is not statistically different in the two group rats. The Cr/protein ratios in the subfractions of the diabetic rats are one to four times lower than those of the normal rats.

Chromium Contents in Subcellular Fractions of Rat Testes

There is still statistically higher Cr content in the testes homogenate of the normal rats than of the diabetic rats ($p < 0.001$), and also in the subcellular fractions ($p < 0.05$). The intracellular results show the higher

Table 4
Chromium Contents in Subcellular Fractions of Rat Testes

Subcellular Fractions	Cr Concentration μg/g		In Total Cell %		Cr/Total Protein μg/g	
	N	D	N	D	N	D
Homogenate	0.21±0.01	0.12±0.01	—	—	2.66±0.18	1.52±0.11
Residue	0.46±0.01	0.19±0.01	8.56	10.9	1.32±0.07	3.48±0.26
Nuclei	0.45±0.01	0.22±0.01	14.7	11.7	2.99±0.17	1.08±0.07
Mitochondria	0.63±0.01	0.36±0.01	8.03	8.50	2.06±0.11	1.10±0.06
Lysosome	0.58±0.01	0.34±0.01	3.68	3.89	1.50±0.08	0.87±0.05
Microsome	0.41±0.01	0.20±0.01	2.87	3.26	1.23±0.07	0.74±0.05
Cytosol	0.32±0.01	0.12±0.01	62.2	61.8	3.59±0.18	1.72±0.14

Notes: The testes of six rats were homogenized together and then separated into subcellular fractions. The chromium measurement error is less than 5%. N—normal rats; D—diabetic rat.

Cr/protein values in the normal rats than in the diabetic rats, too. The cytosol has the highest value for Cr, whereas the rest of the Cr is primarily contained in nuclei and mitochondria. (See Table 4.)

Chromium Contents in Subcellular Fractions of Rat Kidney

The Cr distribution pattern, either in the homogenate or in the subcellular fractions in kidney of the two group rats, is not much different from its pattern in the pancreas and testes. The Cr contents in the subcellular fractions of kidney of the normal rats are somewhat higher than those of the diabetic rats, and so also are the results expressed as Cr/protein. Almost 53–55% of total Cr is accumulated in cysotol, whereas the rest is retained in the nuclei and mitochondrial fractions. (See Table 5.)

DISCUSSION

It has been suggested *(8)* that the essential trace elements in different organ systems often show a normal function only within narrow concentration limits and that deviations from the normal concentrations are often signals of disease.

In this study, our results show evidence of differences of the Cr distribution in various organs and subcellular fractions between the normal and diabetic rats, although their Cr treatment is the same.

Our previous study *(3)* indicated that diabetic rats contained less Cr in their bodies than did normal rats, because of their higher Cr excretion amounts in the urine. In this study, the analysis of Cr contents in the

Table 5
Chromium Contents in Subcellular Fractions of Rat Kidney

Subcellular	Cr Concentration μg/g		In Total Cell %		Cr/Total Protein μg/g	
Fractions	N	D	N	D	N	D
Homogenate	1.28±0.01	1.09±0.01	—	—	6.25±0.32	4.76±0.24
Residue	1.81±0.02	1.01±0.01	6.57	8.40	3.84±0.20	3.16±0.16
Nuclei	1.65±0.02	1.17±0.01	15.6	19.4	3.95±0.20	3.18±0.16
Mitochondria	2.00±0.02	1.55±0.02	12.7	8.64	5.95±0.31	4.52±0.23
Lysosome	1.80±0.02	1.53±0.02	6.96	6.91	7.73±0.40	5.75±0.30
Microsome	1.35±0.02	0.98±0.01	2.81	3.00	4.08±0.21	3.14±0.16
Cytosol	0.72±0.01	0.55±0.01	55.4	53.6	8.36±0.43	6.54±0.34

Notes: The kidney of six rats were homogenized together and then separated into subcellular fractions. The chromium measurement error is less than 5%. N—normal rats; D—Diabetic rat.

liver, kidney, pancreas, and testes of the two group rats again reconfirmed our previous results.

Liver is an important metabolic organ where the metabolism of protein, carbohydrate and lipid, and so forth in men and animals occurs. The nutrients carried by blood are first sent to the liver, and after various physiological transformations, they are partly stored in it or are transported out of it. When Cr enters the liver cell, it is mainly accumulated in the nuclei fraction, which contains the most abundant DNA in all of the organelles. Therefore, it is suggested that chromium has a great tendency to combine with DNA. In fact, there are many studies that indicated Cr(III) to be strongly associated with DNA and protein to form complexes as Cr(III)–protein–DNA *(9,10)*. Additionally, DNA in nuclei is mainly combined with protein, forming a so-called nuclear protein. That is why the nuclei fraction was the highest value of all the subcellular fractions when Cr contents is expressed as the Cr/protein ratio. Mitochondria, lysosome, and microsome are the organelles that have the functions of digestion, and degradation, and so forth in the cell. Mitochondria is the final site for a variety of biosynthetic processes, including carbohydrate, amino acid, and fatty acid oxidation, in which insulin is a necessary hormone for these metabolisms. The microsome obtained by the differential centrifugation contains much smooth endoplasmic reticulum *(11)*, which is probably involved in the metabolism of fats, degradation of glycogen, and the synthesis of phosphoric acid and cholesterol. In this experiment, we use alloxan to induce a diabetic rat model, because alloxan can destroy the β cells of the pancreas and create inadequate secretion of insulin in the body. Therefore, significantly high Cr contents ($p<0.05$) in the liver mitochondrial and microsomal fractions of diabetic rats compared with those in the normal rats might

suggest the physiological functions of Cr participating in glucose or lipid metabolism to compensate the low level of insulin in the body of the diabetic rats. Some previous studies showed that insulin reacted with mitochondria through the formation of a sulfhydryldisulfide linkage and this reaction was enhanced by the presence of Cr(III) *(12,13)*.

The pancreas, testes, and kidney have different physiological functions from liver, so the organic components in them are not the same. There are many sorts of digestive enzyme in the pancreas (e.g., trypsin, amylopsin, and steapsin, etc.). Most of these enzymes are involved in the pancreatic cytosol. The fact that Cr is mainly accumulated in the pancreatic cytosol fraction suggests that Cr might combine with proteins in the pancreas. Nevertheless, the Cr/protein ratios in the pancreatic subcellular fractions of the diabetic rats are one to four times lower than in the normal rats, which indicates that Cr(III) in the pancreas of the diabetic rat did not show special accumulation or combination. That might suggest that Cr could not play its biological function via accumulation in the pancreas and combination with insulin. Hence, it is more likely that Cr, as a cofactor for the peripheral action of insulin, enhances the sensitivity of insulin receptors or plays a synergetic role with insulin-sensitive tissues.

The behavior of Cr(III) in the testes and kidney subcellular fractions deciphers that Cr(III) is possibly combined with protein as Cr–protein complexes and retained in them. Further study of Cr–protein speciation in animal bodies is in progress in our laboratory.

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