Neutron Activation Analysis of Normal and Cadmium Injected Rat Liver Using Ammonium Pyrrolidinedithiocarbamate Extraction

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A radiochemical group separation using APDC extraction was applied to the neutron activation analysis of normal and cadmium injected rat liver. In order to optimize determinations of induced radionuclides with various half lives, the gamma-ray spectra were obtained after various decay times. Eight elements, Cd, Co, Cu, Fe, Mn, Mo, Se and Zn, were determined from rat liver samples within 12 day after irradiation. Although Cd, Cu and Mo in normal rat liver could not be detected by nondestructive method, they could be determined after the extraction.

The tendency was seen that the concentration of all the elements as mentioned above are increased by cadmium injection. In particular, zinc and copper exhibited high values.

Key Words: neutron activation analysis, rat liver, ammonium pyrrolidinedithiocarbamate, solvent extraction, cadmium

1. Introduction

In recent years, the development of Ge(Li) detectors with high resolution has made the instrumental determination very useful in neutron activation analysis of biological materials. About thirty elements can be determined by the nondestructive method, but some elements can not be detected due to the disturbance of sodium, phosphorus or bromine present in biological materials. In order to remove those disturbing elements, many post-irradiation radiochemical group separation methods with some chemical separation, such as precipitation, ion-exchange, solvent extraction and others, have been proposed. The solvent extraction method using organic chelate reagents become more effective due to its rapidity and selectivity. Ammonium pyrrolidinedithiocarbamate (abbreviated as APDC) is one of the most important reagents among generally applicable reagents, such as oxine, dithizone, cupferron or others. The APDC chelate compounds of about thirty elements have been known in which transition elements important in biological problems are all included. The application of a new radiochemical group separation method using APDC reagent in the extraction procedure in neutron activation analysis has recently been reported by Kusaka et al. The validity of the method has been demonstrated by analyzing the NBS standard reference materials.

In the present study, this method has been applied to neutron activation analysis of normal and cadmium injected rat liver. The eight elements, Cd, Co, Cu, Fe, Mn, Mo, Se and Zn, are determined from rat liver samples. In particular, the analytical results of Cd, Cu and Mo in normal rat liver, which could not be detected by the nondestructive method reported previously, can be obtained.

2. Experimental

2.1 Preparation of the samples and standards
Six male rats of Wistar strain about 200 g were divided into two groups. One group was killed without cadmium injection. The other group was injected subcutaneously in the interscapular region with 0.5 mg Cd/kg body weight as an isotonic saline solution of cadmium chloride of 0.1 mg Cd/ml. Injections were made three times on alternate days. The all animals
were sacrificed by decapitation. The liver of these rats were removed, washed in distilled water, lyophilized and crushed by agate mortar.

NBS Bovine Liver (SRM-1577) was used as a multielemental irradiation standard, because the concentrations of seven elements, Cd, Co, Cu, Fe, Mo, Se and Zn, in Bovine Liver are higher than in Orchard Leaves.

The all samples and standards were dried in air oven at 85°C for 4 hours prior to irradiation, and 500 mg of each sample was packed in polyethylene sheets.

2.2 Neutron irradiation

Samples and standards were contained in a polyethylene capsule and neutron irradiated for one hour at the pneumatic irradiation facility of the Kyoto University Reactor with an estimated thermal neutron flux of $2.3 \times 10^{13} \text{n cm}^{-2} \text{s}^{-1}$.

2.3 Radiochemical separation procedure

Each of the samples and standards irradiated was transferred into a 200 ml distillation flask and one ml of the carrier solution was added to it. The carrier solution is a mixture of Cu, Mn, Fe, Zn and Cd solutions, and contains 20 μg/ml of each element. The samples were mineralized in a wet way by a mixture of 2 ml of conc. H2SO4 and 3-5 ml of conc. HNO3. After removal of the excess HNO3 by evaporation, the bromine activity was completely removed by adding one ml of 10% KBr solution and re-evaporating to SO3 fuming. All the distillate was introduced into a 20 ml of 5N NaOH solution through a Liebig condenser to absorb halogens.

Forty ml of H2O, 5 ml of 2M ammonium acetate solution and 5 ml of 2% APDC solution were added to the resultant sample solution. And then the solution was adjusted to 4.0 pH by the addition of NH4OH or HCl, and extracted by 10 ml of CHCl3. Aqueous Phase was again extracted with 10 ml of CHCl3. Organic Phases were combined and shaken with 11 ml of an aqueous solution containing 10 ml of 1M NaCl and one ml of 2% APDC solution to wash out any remaining sodium activity. Next, Aqueous Phase was adjusted to 7.0 pH with NH4OH and the manganese activity was separated into CHCl3 phase by the same procedure as mentioned above. Both Organic Phases were combined and then evaporated on a hot plate to dryness. The residue was dissolved in one ml of conc. HNO3, and the solution volume was made up to 7 ml by adding 1N HCl for the radioactivity measurement.

2.4 Measurement of gamma activity

Sample solutions after the radiochemical separation were counted with a coaxial Ge(Li) detector (FWHM 2.0 keV at 1.332 MeV) coupled to a 4096 channel pulse-height analyzer. For the purpose of comparison, one of the irradiated samples was also counted without any radiochemical separation. The gamma activity was measured for 3,000, 6,000, 10,000 and 10,000 seconds after a decay of 10 hours, 3 days, 12 days and 30 days, respectively.

3. Results and Discussion

3.1 A comparison of gamma-ray spectrum

Typical gamma-ray spectra obtained from normal and cadmium injected rat liver with and without radiochemical separation are shown together with that of an aliquot of the aqueous phase after the radiochemical separation in Fig. 1, 2 and 3.

In Fig. 1, photo-peaks of 69mZn, 65Zn, 64Cu and large photo-peaks of 847 keV and 1811 keV from 56Mn in the radiochemically separated samples are identified. The peak of 115Cd(115mIn; daughter nuclide of 115Cd) is also obvious in Cd injected rat liver. In the nondestructive method, large peaks of 24Na and 42K and a small peak of 82Br are observed. It is shown in Fig. 1 that 24Na and 42K were removed completely by this radiochemical separation method and that manganese was able to be determined.

In Fig. 2, longer-lived nuclides such as 58Fe, 65Zn and 60Co, which can be barely determined after a 30-day decay on the nondestructive method, are detected in the samples separated. The peaks of 99Mo (740 keV and 780 keV) and 99mTc (140 keV), which is daughter nuclide of 99Mo, are also detected. The total count of 115Cd(115mIn) is higher than that in Fig. 1.
peaks of $^{69m}$Zn (439 keV) and $^{64}$Cu (1346 keV) are observed again. On the other hand, any other nuclides, except $^{24}$Na, $^{42}$K and $^{82}$Br, are not detected in the normal rat liver samples by the nondestructive method. But in the Cd injection samples, the peaks of $^{114m}$In (115 Cd) are detected. $^{76}$As is observed in both organic and aqueous phase after the radiochemical separation, so As cannot be determined. From Fig. 2, Cu, Cd and Mo are able to be determined.

It can be seen in Fig. 3 that background spectrum of low energy region due to bremsstrahlung radiation of $^{32}$P betaray is decreased by the extraction, because phosphorus is removed by this radiochemical separation method. It is possible to measure the peaks of $^{75}$Se. In the gamma-ray spectrum of the nondestructive method, many peaks of $^{82}$Br are observed again, and also the peaks of $^{75}$Se, $^{134}$Cs, $^{86}$Rb, $^{59}$Fe, $^{65}$Zn and $^{60}$Co are detected. On the other hand, $^{134}$Cs and $^{86}$Rb can be detected in the aqueous phase sample.

After a decay of 30 days, the gamma-ray spectra obtained from cadmium injected rat liver sample become similar to those from normal rat liver sample, because all peaks of Cd disappear. The longer-lived nuclides can be determined from the nondestructive spectrum, but counting error, especially at low energy side, is large owing to the bremsstrahlung of $^{32}$P.

It may be concluded from the above discussion that the gamma-ray spectra of the separated fraction can be measured on the day of the irradiation for Mn; after a 3-day decay for Cu, Cd, Mo, Co, Fe and Zn; and after a 12-day decay for Se. Thus, seven elements other than Se can be determined within only 3 days after neutron irradiation, while a 30-day decay is required to determine these elements by the nondestructive method.

It can also be concluded that by this separation biologically essential or toxic
transition elements are extracted into the organic phase leaving alkali elements, in the aqueous phase.

3-2 Analysis of normal and cadmium injected rat liver

The results of the eight elements such as Cd, Co, Cu, Fe, Mn, Mo, Se and Zn are given in Table 1. The present results for normal rat liver are compared with our former results obtained by the nondestructive method. By the use of radiochemical separation using APDC extraction, Cd, Cu and Mo in the normal rat liver can be determined. The values of Fe, Mn, Se and Zn are in fair agreement with those by the nondestructive method, but the agreement in Co is not so good.

On the other hand, in the cadmium injected rat liver, the above eight elements were also determined. It has been found that there is a tendency for the concentration of the eight elements to increase caused by cadmium injection. In particular, the relative concentrations of zinc and copper exhibit high values. The increase of zinc and copper concentrations is considered to have some relation with the production of cadmium binding protein (metallothioneine) in liver by injection of cadmium.

Further study of the metallothioneine, which binds with many metal elements, by neutron activation analysis should be required in studying the physiological and pathological roles of trace elements in biological materials.

References

要 旨

Ammonium Pyrrolidinedithiocarbamate 抽出を用いる正常ラットおよびCd 投与ラット肝臓の中性子放射化分析

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APDC 抽出法を使用する放射化学的グループセパレーション法を正常ラットおよび Cd 投与ラット肝臓の中性子放射化分析に応用した。種々の半減期をもつ誘導核種の最適な測定のために，種々の減衰時間の後に得られた γ 線スペクトルを検討した。

照射後12日以内に，ラット肝臓試料中の Cd, Co, Cu, Fe, Mn, Mo, Se, Zn の8元素を測定した。非破壊法では検出されなかった正常ラット肝臓中の Cd, Cu, Mo の分析値を得ることができた。

Cd を投与することによって，上記のすべての元素濃度は増加する傾向がある。とくに Zn と Cu は高い値を示した。