Synthesis of AM15C5 Bonded Merrifield Peptide Resin and its Separating Properties

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A 2-aminomethyl-15-crown-5 (AM15C5) bonded Merrifield peptide resin was synthesized. The capacity of this crown ion exchanger was 2.25 meq/g of dry resin. The heavier isotopes of magnesium were enriched in the solution phase, while the lighter isotopes were enriched in the resin phase. The hydration and isotope mass effects dominated over those of the complexation and properties of the exchanger. The separation factors of $^{24}Mg^{2+}/^{25}Mg^{2+}$, $^{24}Mg^{2+}/^{26}Mg^{2+}$, and $^{25}Mg^{2+}/^{26}Mg^{2+}$ isotope pair fractionations were 1.0012, 1.0023, and 1.0011, respectively.

Introduction

Macrocyclic polyethers and their analogues have the remarkable property of complexation with cations, especially, alkali and alkaline earth metal ions [1]. Therefore, their unique ability to form stable complexes with various cations has been used to isotope separation for alkali and alkaline earth metal ions [2]. Recent investigations on separation of isotopes by cyclic polyethers have been performed due to the significant isotope effects [3–12]. A more stable 1:1 complex is formed when the cation has an ionic diameter fitted to the cavity size of the crown ether. The cavity diameter of AM15C5 (15-crown-5) is 17–22 nm [13], and the ionic diameter of Mg^{2+} is 13–17.4 nm [14], respectively. This may produce a stable complex formation and hence isotope effect. The smaller the crown ether ring, the larger the separation factor of lithium isotopes for the macrocycles investigated [15]. Nishizawa *et al.* [15] reported that the separation factors of lithium isotopes at 0 °C were 1.057 for 12-crown-4, 1.042 for benzo-15-crown-5, 1.041 for lauryloxymethyl-15-crown-5, 1.043 for tolyloxymethyl-15-crown-5, and 1.024 for dicyclohexano-18-crown-6. These values suggest that the larger separation factor is obtained when the macrocycles have no substituted groups and the more stable complex is formed [13,15]. Nishizawa *et al.* [11] obtained a separation factor of 1.0112 as a maximum value for the $^{24}Mg^{2+}/^{26}Mg^{2+}$ isotope pair by a liquid-liquid extraction system using the dicyclohexano-18-crown-6 (DC18C6). Recently, Kim *et al.* [12] reported that elution chromatographic separation of magnesium isotopes was investigated by chemical ion exchange with the synthesized 1,7-dioxa-4,10,13-triazacyclopentadecane-4,10,13-tris(Merrifield peptide resin). They obtained values of the separation factor ranging from 1.009 to 1.030. In this work, magnesium isotope separation was investigated using a AM15C5 bonded Merrifield peptide resin by ion exchange elution chromatography.

Results and Discussion

The AM15C5 resin was prepared by the method given in the literature [16,17]. The synthetic route of the AM15C5 bonded Merrifield peptide resin is shown in Scheme 1. The degradation of the Merrifield resin began at 290 °C and ended at 405 °C, and T_{max} was 360 °C. The capacity of the AM15C5 resin was determined by the method given in the

literature [18]. The ion exchange capacity of the AM15C5 resin was 2.25 meq/g dry resin. For the determination of the distribution coefficient, a batch method was employed [19]. The distribution coefficient of magnesium ion on the resin was measured by changing the concentration of NH_4Cl solution from 1.0×10^{-3} M to 4.0 M using a batch method. The distribution coefficients were calculated using eq. (6). As shown in Fig. 1, the distribution coefficients of magnesium ions on the AM15C5 resin increased in a non-linear manner with increasing concentration over a range from 1.0×10^{-3} M to 4.0 M NH_4Cl solution.

The column operation was performed with 2.5 M NH_4Cl solution (K_d = 179) as eluent at 20 °C. The elution time increased with increasing the distribution coefficient due to the high adsorption of ions on the resin phase in the column. For this reason, as expected, the eluents having distribution coefficients ranging from 30 to 300 were used to separate isotopes in our laboratory. From the elution curve, the number of theoretical plates in the column was calculated by eq. (1):

$$N = 8 \cdot \left(\frac{V_{max}}{\beta} \right)^2 \quad (1)$$

where V_{max} is the peak elution volume, β the band width at the concentration $C = C_{max}/e$, and C_{max} the concentration of solute at the maximum peak height of the elution curve. The e means base of natural logarithms.

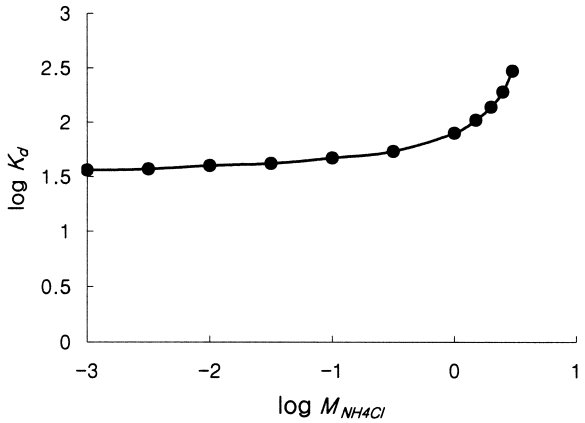


Fig. 1. Plot of log K_d for magnesium ions on the AM15C5 bonded Merrifield peptide resin as a function of NH_4Cl solution concentration.

From the experimental data, it appears that the isotope exchange reaction can be represented by eqs (2–4):

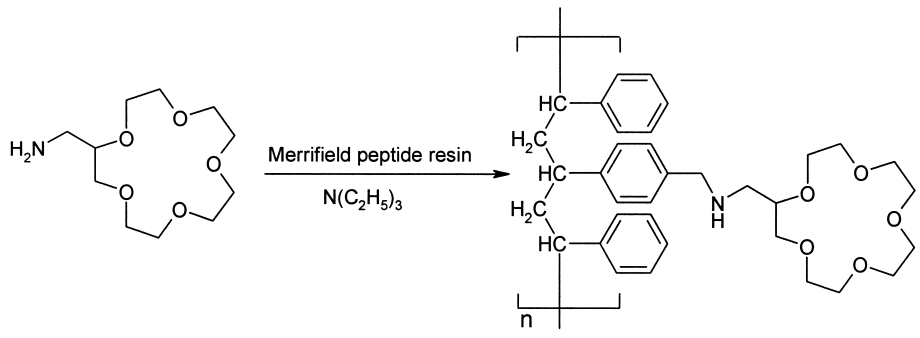
$$^{24}Mg^{2+}_{solution} + {}^{25}Mg^{2+}_{resin} \rightleftarrows {}^{24}Mg^{2+}_{resin} + {}^{25}Mg^{2+}_{solution} \quad (2)$$

$$^{25}Mg^{2+}_{solution} + {}^{26}Mg^{2+}_{resin} \rightleftarrows {}^{25}Mg^{2+}_{resin} + {}^{26}Mg^{2+}_{solution} \quad (3)$$

$$^{24}Mg^{2+}_{solution} + {}^{26}Mg^{2+}_{resin} \rightleftarrows {}^{24}Mg^{2+}_{resin} + {}^{26}Mg^{2+}_{solution} \quad (4)$$

The subscripted symbols such as solution and resin refer to the NH_4Cl solution and AM15C5 resin phases in this chemical isotope exchange.

The separation factors of magnesium isotopes were calculated by the Glueckauf theory [20,21]. The data were plotted on probability paper where the abscissa was a probability scale and the ordinate was a linear scale. The local enrichment factor (log R) was the ordinate and the fraction of the eluted mixture ($\Delta m/m$) was the abscissa. This



Scheme 1. Synthesis of the AM15C5 resin.

gave a linear plot. The slope of the straight line obtained will be $\varepsilon N^{1/2}$, and separation factor is ε+1. The separation factor, α, was determined from the slope of a least squares line drawn through the points as shown in Fig. 2.

In this experiment, the magnesium isotope separation factors for $^{24}Mg^{2+}/^{25}Mg^{2+}$, $^{24}Mg^{2+}/^{26}Mg^{2+}$, and $^{25}Mg^{2+}/^{26}Mg^{2+}$ were obtained as values of 1.0012, 1.0023 and 1.0011, respectively. These values are larger than the value determined by Aaltonen [22], who carried out an elution chromatographic separation of magnesium isotopes with a strongly acidic cation exchanger, Dowex 50W × 8, and found that the value of the separation factor for the $^{25}Mg^{2+}/^{26}Mg^{2+}$ pair was 1.00016. However, the values of our experimental results are lower than those obtained by Kim *et al.* [12] and Nishizawa *et al.* [11]. These phenomena can be explained by the fact that different experimental materials and methods were employed. Nishizawa *et al.* [11] investigated the extraction method using organic phase including dicyclohexano-18-crown-6 from magnesium chloride.

On the other hand, Kim *et al.* [12] investigated the elution chromatographic separation using a triazacrown (*N,N,N,O,O*-15-crown-5) by ion exchange. This triazacrown has no substituents but AM15C5 bears a 2-aminomethyl group. The side chain of the crown ethers changes the stability constant of the complex and the separation factor. The larger the side chain, the smaller a separation factor of isotopes was observed [15]. This effect may have reduced the isotope effect in our experiment. Aaltonen [22] reported that the heavier isotope of magnesium was enriched in the front parts of magnesium adsorption bands formed in chromatographic separation columns. Jepson *et al.* [3] and Fujine *et al.* [23] also reported that the heavier isotopes were preferentially enriched in the solution phase of chromatography using crown ethers. These results agree well with our work. On the other hand, Kim *et al.* [24] and Heumann *et al.* [25] reported that the heavier isotopes were preferentially enriched in the resin phase. Lee [26] has shown that the metal ion species in the resin phase is less hydrated than the metal ion species in the solution phase. This contributes to a difference in bonding and subsequent enrichment of the lighter isotopes in the resin phase. These phenomenona are consistent with our system. The enrichment factor ($\varepsilon = \alpha$-1) for isotopes separated by ion exchange or extraction chromatography depended upon the mass of the isotope as well as the difference in the masses of the isotope pairs [26]. Isotopes within approximately the same mass range may have an increase or decrease in factor due to ion complexing, but the mass effect is more significant [26]. In the resin phase, where the water molality is reduced and the water structure is disrupted by the presence of the organic matrix, magnesium ions are less strongly hydrated, and hence, less strongly bonded, than in the dilute exterior solution surrounding the resin [27,28]. The heavier, smaller isotope, which tends to concentrate preferentially in the more strongly bonded species, [14,28–30] thus favors the aqueous phase, and the lighter isotope is displaced into the resin phase. The heat of hydration of eluting NH_4^+ ion is relatively small [31], but the counter Cl^- ion is the significant structure breaker [32–34], and this may be contributed to the isotope effect [32–35] in this experiment. In conclusion, the magnesium isotope separation system in this work can, therefore, be explained by the fact that, the hydration and isotope mass effects were dominant than those of the complexation and properties of the crown ion exchanger.

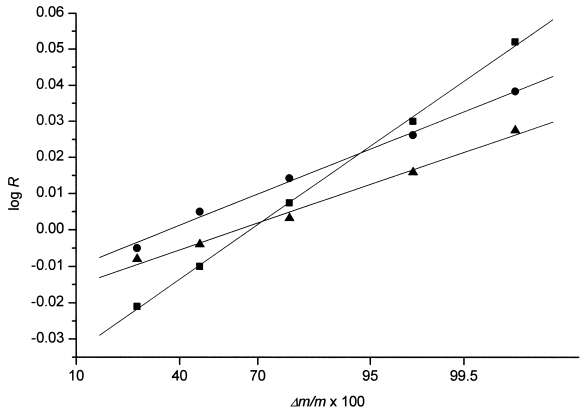


Fig. 2. Enrichment of magnesium isotopes by cation exchange elution chromatography, ● : $^{24}Mg^{2+}/^{25}Mg^{2+}$; ■ : $^{24}Mg^{2+}/^{26}Mg^{2+}$; ▲ : $^{25}Mg^{2+}/^{26}Mg^{2+}$.

Experimental Section

Synthesis of 2-aminomethyl-15-crown-5 (N – Merrifield peptide resin) [16,17]

The AM15C5 was put into a 500 ml three necked round-bottom flask, equipped with a condenser, addition funnels, and moisture protector, were placed dry DMF (200 ml) and triethylamine (2 ml). The oily residue, dissolved in DMF (50 ml), was added slowly. After the mixture was stirred for 72 h at 88–90 °C, the residue was washed with water and methanol to leave a yellow powder. The C-Cl stretching vibration of the Merrifield peptide resin was found at 690 cm^{-1} in an IR spectrum. In the IR spectrum of the yellow product, the C-Cl absorption (KBr, 690 cm^{-1}) was lower in intensity. This indicates a N-C bond formation between a nitrogen atom of the crown and the Merrifield peptide resin.

Reagents and apparatus

$MgCl_2$ and NH_4Cl were purchased from Sigma Chemical Co., USA. An atomic absorption spectrophotometer(AAS, Hitachi Z-8000) was used to determine the magnesium ion concentration in the solution. Measurement of the magnesium isotope ratio was carried out using a thermal ionization mass spectrometer (Finnigan MAT 262) with a rhenium double filament. An amount of 1.0–2.0 μg of magnesium was loaded on an evaporation filament. Ionization was then performed by passing a heating electric current through the ionization filament. After the ion beam intensities of $^{24}Mg^{2+}$, $^{25}Mg^{2+}$, and $^{26}Mg^{2+}$ became sufficiently high, the $^{24}Mg^{2+}$, $^{25}Mg^{2+}$, and $^{26}Mg^{2+}$, mass peaks were repeatedly recorded. The mass scanning was repeated several times in a block, and several blocks were recorded as one measurement. The mole fraction of $^{24}Mg^{2+}$, $^{25}Mg^{2+}$, and $^{26}Mg^{2+}$ of each feed solution was an average of three times in our measurement.

Ion exchange capacity [18]

The cation exchanger was transformed into the H-form by slow treatment with 1.0 N HCl (~ 1 l). Subsequently, it was washed to neutrality with distilled water, and dried in air. Of this quantity, 1.000 ± 0.005 g was weighed into a dry 250 ml Erlenmeyer flask containing exactly 200 ml of 0.1 N NaOH with 5% aq. NaCl, and was allowed to stand overnight. The amount of 1.0 g of ion exchanger of the same material was separately weighed into a weighing bottle, dried at 110 °C overnight, and weighed again to determine the percentage of solids. Of the supernatant liquid in the Erlenmeyer flask, 50 ml aliqots were backtitrated with 0.1 N HCl against phenolphthalein. The capacity was then calculated by eq. (5):

$$\text{Capacity (meq/g)} = \frac{\{(200 \cdot \text{normality}_{NaOH}) - 4(\text{ml}_{acid} \cdot \text{normality}_{HCl})\}}{\text{sample weight(g)} \cdot (\%\text{solid}/100)} \quad (5)$$

It represents the total weight of the exchanger in the dry H-form. The resin must be completely in the H-form before weighing of the sample, since difference in equivalent weights of different ions would lead to errors. The standard sodium hydroxide solution was treated with 5% sodium chloride to obtain a complete exchange equilibrium by the excess of sodium ions. A reproducibility of ± 1% can consequently be obtained.

Measurement of distribution coefficients [19]

Each portion of 1.0 g of the AM15C5 bonded Merrifield peptide resin (200 ~ 400 mesh), which had been dried to a constant weight at 60 °C, was weighed out accurately and transferred into a 100 ml polyethylene vial with a polyethylene screw top. Then, 1.0 ml of 0.01 M $MgCl_2$ solution was added, followed by 49 ml of aq. NH_4Cl of the desired concentration to give a final volume of 50 ml. The reaction mixture was subjected to reciprocal shaking at 100 strokes/min for 24 h, and then centrifuged for 5 min at 5000 rev/min. The concentration of magnesium ions in the supernatant was determined using the AA-spectrophotometer. The distribution coefficient, K_d, was calculated by eq. (6):

$$K_d = \frac{(C_{st} - C_{eq}) \cdot V}{C_{eq} \quad m} \quad (6)$$

where C_{st} is the metal ion concentration of the standard solution, C_{eq} the metal ion concentration after equilibrium, V the total volume in ml of the solution, and m the mass in gram of dry resin.

Separation of lithium isotopes

The AM15C5 resin was slurried in 2.5 M aq. NH_4Cl. The slurried resin was packed in a water-jacketed glass column (0.2 cm i.d. × 35 cm height). The temperature was maintained at 20 °C using an water circulator (HAAKE A-80). The concentration of 400 ppm of Mg^{2+} in distilled water was loaded on the top of the resin bed. 2.5 M NH_4Cl solution (K_d = 179) was used as an eluent. The

magnesium feed solution was then passed through the column under gravity flow. The flow rate was controlled by a fine stopcock to be 0.6 ml/h. The effluent was collected as a fraction of 0.1 ml each with an automatic fraction collector (Pharmacia LKB FRAC-100).

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