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The Adsorption of Nucleic Acid Bases by Raney-nickel, -cobalt, and -copper

Keisuke Mita,* Kazuhiko Hotta, and Shoji Watanabe
Osaka Municipal Technical Research Institute, Ogimachi, Kita-ku, Osaka 530

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The order of the relative affinity of adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U) to Raney-nickel, Raney-cobalt and Raney-copper was determined in an alkaline solution by means of a column-chromatographic method. These bases could be placed in these orders: G>A>C>U>T for Raney-nickel, G>C>A>T~U for Raney-cobalt, and A~G~C>T~U for Raney-copper. It was also found that these Raney-metals could be placed in the following order as to ability to distinguish those bases: Raney-nickel≫Raney-cobalt> Raney-copper.

It has been well known that metallic nickel powder can induce lung cancer.[1,2] Some investigators consider that the interaction between DNA and the nickel ion leached from metallic nickel is directly responsible for producing cancer.[1,3] The reactions of nucleic acids, nucleotides, nucleosides, or bases with the nickel ion have, therefore, been studied by many workers.[2,4,5] Although it has been known that the nickel ion interacts with both nucleic acid bases and phosphate portions in DNA, there have been few reports concerning the precise interaction sites of nucleic acids with the nickel ion.

In the present work, we determined the order of the relative affinities of adenine, guanine, cytosine, thymine, and uracil for metallic nickel, cobalt, and copper by means of a column-chromatographic method and tried to estimate the interaction sites of nucleic acid with the metal ions. Raney-nickel (R-Ni), Raney-cobalt (R-Co), and Raney-copper (R-Cu) were used as the metals because of their high surface area and well-known surface properties.[6,7]

Experimental

R-Ni (Ni: 2.5 g), R-Co (Co: 10 g), and R-Cu (Cu: 10 g) were prepared from commercial Raney metal–aluminium alloys (Al/metal: 50 atom%) by leaching out the aluminium with a 13% sodium hydroxide solution for 1 h at 70, 50, and 50 °C respectively. After washing with distilled water, each sample was packed in a 1-cm-diameter column, which was fitted at the bottom with a cotton to retain the sample, to a height of approximately 5, 20, or 20 cm respectively. A 0.2 mol dm^{-3} sodium hydroxide solution containing two or three kinds of bases at 2.0 mmol dm^{-3} was allowed to flow into the column at a rate of 10 ml/h. The effluent was collected by fraction collecter. The concentration of each base in the effluent fractions could then be determined by using the absorption spectra of the fractions and by solving Eq. 1:

$$\begin{aligned}\frac{A_\alpha}{A_\alpha}x + \frac{B_\alpha}{B_\beta}y + \frac{C_\alpha}{C_\gamma}z &= S_\alpha\,,\\ \frac{A_\beta}{A_\alpha}x + \frac{B_\beta}{B_\beta}y + \frac{C_\beta}{C_\gamma}z &= S_\beta\,,\\ \frac{A_\gamma}{A_\alpha}x + \frac{B_\gamma}{B_\beta}y + \frac{C_\gamma}{C_\gamma}z &= S_\gamma\,,\end{aligned} \tag{1}$$

where A_α, A_β, and A_γ are the absorbances of a 0.1 mol dm^{-3} H_2SO_4 or 0.1 mol dm^{-3} NaOH standard solution containing known quantities of base-A at the maximum absorption wavelengths of base-A (α), base-B (β), and base-C (γ) respectively. Moreover B_α, B_β, and B_γ are the absorbances of base-B, and C_α, C_β, and C_γ, those of base-C at the wavelengths of α, β, γ respectively. S_α, S_β, and S_γ are the absorbances of the standard solutions containing a known quantity of the effluent fraction at the wavelengths of α, β, and γ, while x, y, and z are the concentrations of base-A, base-B, and base-C respectively in the fraction.

The uncertainty of the method is determined by mixing these bases in different known proportions and by estimating the base contents in the mixtures by means of Eq. 1. The experimental conditions are shown in Table 1.

Results and Discussion

It has been known that the fraction of the metallic surface is in the range of 55—85% for conventional preparations of Raney-metals, and the residual fraction is considered to be alumina.[7] In order to examine whether or not the nucleic-acid bases can be absorbed by alumina, a 0.2 mol dm^{-3} sodium hydroxide solution containing these bases was allowed to flow into a column packed with γ-alumina. Since the adsorption of these bases by γ-alumina could not be observed, the possibility of adsorption by alumina on Raney-metals can be disregarded.

In the present work, Raney-cobalt or Raney-copper column is packed with an amount four times that of the

Table 1. Experimental conditions

Bases			Standard solution	Wavelength/nm			Uncertainties
A	B	C		α	β	γ	%
Guanine,	Uracil,	Cytosine	0.2 mol dm^{-3} H_2SO_4	248,	260,	276	5
Adenine,	Guanine,	Thymine	0.2 mol dm^{-3} NaOH	269,	274,	291	20
Guanine,	Adenine,	Cytosine	0.2 mol dm^{-3} H_2SO_4	248,	262.5,	276	5
Adenine,	Cytosine		0.2 mol dm^{-3} H_2SO_4	262.5,	276		5
Uracil,	Thymine		0.2 mol dm^{-3} NaOH	284,	291		20

Raney-nickel column, because the amount of the bases adsorbed at saturation for R-Ni is about four times those of R-Co and R-Cu. Therefore, it can be considered that the effluent volume/time·metallic surface area is nearly same in each column.

Figures 1—3 show the breakthrough curves of a guanine–uracil–cytosine mixture for R-Ni, R-Co, and R-Cu respectively. It can be seen from Figs. 1 and 2 that the relative affinities of these three bases for R-Ni and R-Co can be placed in this order: guanine>cytosine>uracil. In the case of R-Cu, on the other hand, the relative affinities of these bases can be placed in this order: guanine∼cytosine>uracil, because the breakthrough curves exhibit no differences between guanine and cytosine, as is shown in Fig. 3. By comparing the breakthrough curves in Figs. 1—3 with each other, it can be seen that these Raney-metals can be placed in the following order of ability to distinguish these bases: R-Ni>R-Co>R-Cu.

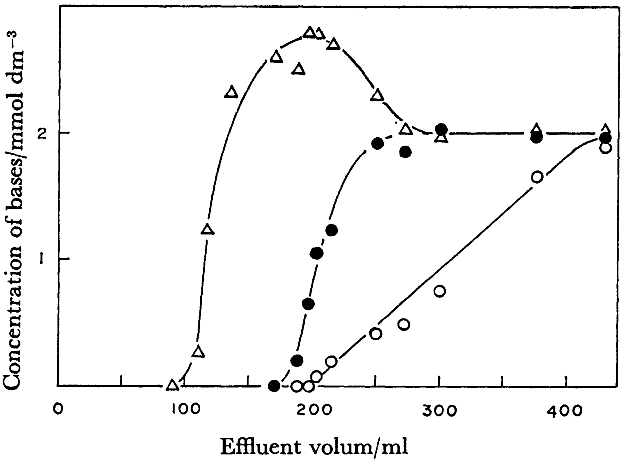

Fig. 1. Breakthrough curves on R-Ni for 0.2 mol dm^{-3} NaOH solution containing guanine (○), cytosine (●) and uracil (△) at the concentration of 2 mmol dm^{-3} respectively.
R-Ni: 2.5 g as nickel, column: 1.0×5 cm, flow rate: 10 ml/h.

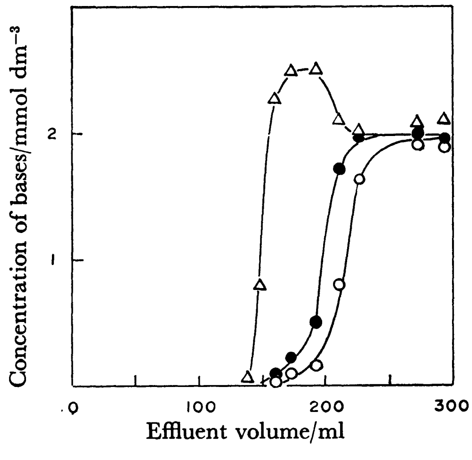

Fig. 2. Breakthrough curves on R-Co for 0.2 mol dm^{-3} NaOH solution containing guanine (○), cytosine (●) and uracil (△) at the concentration of 2 mmol dm^{-3} respectively.
R-Co: 10 g as cobalt, column: 1.0×20 cm, flow rate: 10 ml/h.

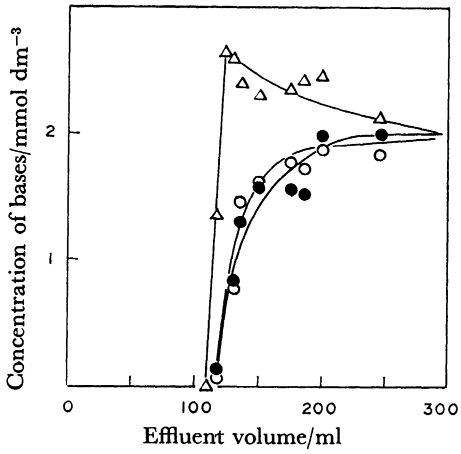

Fig. 3. Breakthrough curves on R-Cu for 0.2 mol dm^{-3} NaOH solution containing guanine (○), cytosine (●) and uracil (△) at the concentration of 2 mmol dm^{-3} respectively.
R-Cu: 10 g as cupper, column: 1.0×20 cm, flow rate: 10 ml/h.

The breakthrough curves of a guanine-adenine-thymine mixture for R-Ni, R-Co, and R-Cu were found to give patterns similar to Figs. 1—3 respectively. It was shown from the breakthrough curves that, among these three bases, thymine exhibits the weakest affinities for Raney-metals. From these results and Figs. 1—3, it appeared that these five bases can be classified into two groups, one of which exhibits relatively strong affinities (guanine, adenine, and cytosine) for Raney-metals, while the other exhibits relatively weak affinities (uracil and thymine).

Figures 4—6 show the breakthrough curves of a guanine–adenine–cytosine mixture for R-Ni, R-Co, and R-Cu respectively. It appears from Fig. 4 that the relative affinities of these three bases for R-Ni can be placed in this order: guanine>adenine>cytosine. On the other hand, it can be seen from Fig. 5 that the relative affinities of these bases for R-Co can be placed in this order: guanine>cytosine>adenine. It may be noted that a reversed order of adenine and cytosine is observed for R-Co. This difference between R-Ni and R-Co was also confirmed by the breakthrough curves of the cytosine–adenine mixture, as shown in Fig. 7. In the case of R-Cu, however, no differences in these three bases could be detected, as is shown in Fig. 6.

Figure 8 shows the breakthrough curves of a uracil–thymine mixture for R-Ni, R-Co, and R-Cu. It appears that the relative affinities of these two bases can be placed in this order: uracil>thymine for R-Ni, while in the case of R-Co and R-Cu no differences between the relative affinities of these two bases was observed within the limits of experimental error.

In conclusion, the present experiment has made it obvious that the relative affinities of these five bases can be placed in these orders: guanine>adenine>cytosine>uracil>thymine for R-Ni, guanine>cytosine>adenine>thymine∼uracil for R-Co, and adenine∼guanine∼cytosine>thymine∼uracil for R-Cu. It is

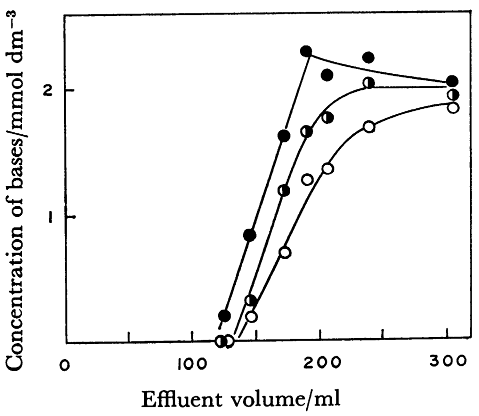

Fig. 4. Breakthrough curves on R-Ni for 0.2 mol dm^{-3} NaOH solution containing adenine (◑), guanine (○) and cytosine (●) at the concentration of 2 mmol dm^{-3} respectively.
R-Ni: 2.5 g as nickel, column: 1.0×5 cm, flow rate: 10 ml/h.

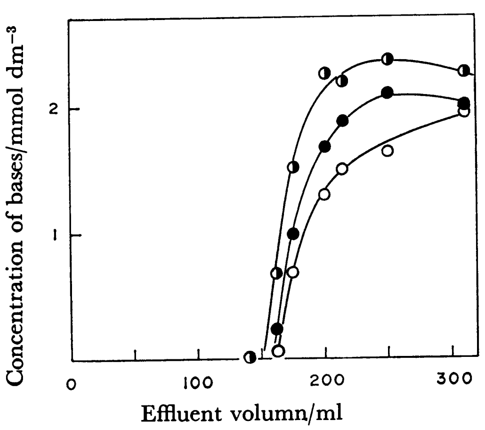

Fig. 5. Breakthrough curves on R-Co for 0.2 mol dm^{-3} NaOH solution containing adenine (◑), guanine (○) and cytosine (●) at the concentration of 2 mmol dm^{-3} respectively.
R-Co: 10 g as cobalt, column: 1.0×20 cm, flow rate: 10 ml/h.

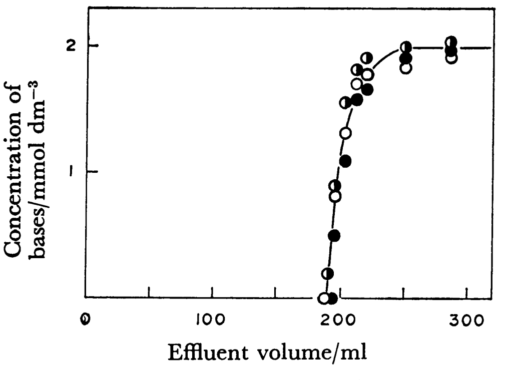

Fig. 6. Breakthrough curves on R-Cu for 0.2 mol dm^{-3} NaOH solution containing adenine (◑), guanine (○) and cytosine (●) at the concentration of 2 mmol dm^{-3} respectively.
R-Cu: 10 g as cupper, column: 1.0×20 cm, flow rate: 10 ml/h.

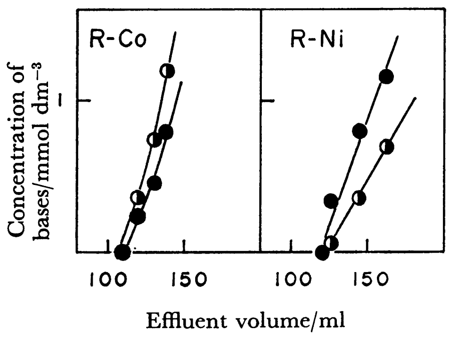

Fig. 7. Breakthrough curves on R-Ni and R-Co for 0.2 mol dm^{-3} NaOH solution containing adenine (◑) and cytosine (●) at the concentration of 2 mmol dm^{-3} respectively.
R-Ni: 2.5 g as niekel, 1.0×5 cm column, R-Co: 10 g as cobalt, 1.0×20 cm column, flow rate: 10 ml/h.

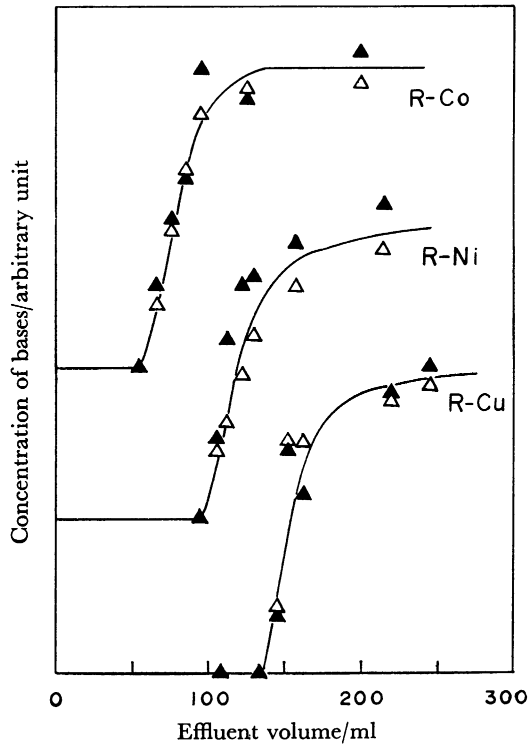

Fig. 8. Breakthrough curves on R-Ni, R-Co, and R-Cu for 0.2 mol dm^{-3} NaOH solution containing thymine (▲) and uracil (△) at the concentration of 2 mmol dm^{-3} respectively.
R-Ni: 2.5 g as nickel, R-Co: 10 g as cobalt, R-Cu: 10 g as cupper, flow rate: 10 ml/h.

also obvious that the ability of Raney-metals to distinguish these bases can be placed in this order: R-Ni>R-Co>R-Cu.

It has been reported that Cu(II),[8–13] Mn(II),[14,15] and Co(II)[14] ions bind mainly to guanine among the bases in nucleic acids. It has also been reported that the antitumor *cis*-dichlorodiammineplatinum(II) complex indicates a preferential binding to guanine,[16–20] followed by adenine[18,21] in DNA. It has also been established that the Ni^{2+}–AMP complex is far more stable than Co^{2+}–AMP complex.[22] On the other hand, the formation of a stable Co^{2+}–CMP complex has been reported.[23,24] Such similarities of behavior between Raney-metals and their metal ions may suggest that the order of the relative affinities of these bases for the nickel ion is similar to that for Raney-nickel. It would also be interesting to determine whether or not the large ability of Raney-nickel to distinguish these bases is reflected by that of the nickel ion.

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