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Equilibrium analysis of reactions between aromatic anions and nonionic surfactant micelles by capillary zone electrophoresis

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Abstract

Separability of some positional isomers of aromatic anions by capillary zone electrophoresis was improved by adding nonionic surfactants to a migrating solution. Eleven kinds of aromatic anions, including positional isomers, were used as analytes, and Brij-35, Brij-58 and Brij-78 were investigated as nonionic surfactants to form micelles, where hydrophobicities are different from each other. Increasing the concentration of the surfactants developed the separability of the anionic isomers. The interaction between the anions and the nonionic surfactant micelles is also investigated through the change in the electrophoretic mobility, and the binding constants are determined. Apparent electrophoretic mobility of the anions decreased with increasing concentrations of the nonionic surfactants. The decrease in the mobility, as well as the binding constant, was larger in the monovalent anions than in the divalent anions, which indicates that the interaction or reactivity of the monovalent analytes is higher than that of the divalent analytes. The reactivity of each anion was almost identical even when the kinds of the surfactants were changed, suggesting that the hydrophobicity of the polyoxyethylene group in the surfactant would have the main role for binding the analytes. The reactivity tendency among the positional isomers was almost similar to that in ion association-capillary zone electrophoresis using tertabutylammonium ion as a pairing ion. The results obtained in this work suggest that the anions are bound to the micelles by the hydrophobic interaction between analyte anions and the polyoxyethylene moiety of the surfactant micelles. Changes in the fluorescence intensity of the anions were also investigated; the results can explain well the mobility changes of the analytes.

Keywords: Positional isomers; Anions, aromatic; Carboxylic acids; Naphthalenesulfonates; Surfactants

1. Introduction

Micellar electrokinetic chromatography (MEKC) with anionic surfactants has been proved to be a powerful technique for the separation of non-charged substances in capillary zone electrophoresis (CZE) [1,2]. In addition to the anionic surfactants, zwitterionic surfactants [3], cyclodextrin [4,5], and organic solvents [6,7]have been used for the separability improvement in combination with the anionic surfactants. Nonionic surfactants were also examined as modifying reagents for the MEKC separations [8–11]. However, the MEKC methods using nonionic surfactants have mainly been focused on the separation of non-charged analytes.

Recently, Matsubara and Terabe demonstrated the separability improvement of anionic dansylamino acids by a MEKC method with nonionic surfactants [12]. In the method, the hydrophobicity of analytes was considered to contribute to the distribution of analytes between the micelle and the water. The method also has a prominent advantage that high concentrations of the surfactants are allowed without

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any increase in the electric current. Nonionic surfactants were also used for the formation of in situ charged micelles [13], for the partial filling technique [14], and for the separation of tetracycline antibiotics [15].

Although the MEKC separation of ionic substances using nonionic surfactants possesses several advantages, it is essential and useful to clarify the interaction between the analytes and the surfactants in the migration systems for designing the prominent separation system.

In this study, the authors aimed at characterizing the interaction when some aromatic anions are used as model analytes; the degree of the interaction was analyzed through the change in electrophoretic mobility of the analyte anions. The reactivity of analyte anions investigated by mobility measurement agreed well with results obtained in fluorescence intensity measurements. The hydrophobic properties of the surfactants were also compared with each other through mobility change and binding constants.

2. Experimental

2.1. Apparatus

An Applied Biosystems (Foster City, CA, USA) 270A-HT capillary electrophoresis system with a UV detector was used. A fused-silica capillary purchased from GL Sciences (Tokyo, Japan) was attached to the system; the size of the capillary was 72 cm (50 cm effective length from the sample injection point to the UV detector)×50 μm I.D. A Hitachi (Tokyo, Japan) D-2500 Chromato-Integrator was used for recording the electropherograms. A Hitachi 650-10S fluorescence spectrophotometer was used for fluorometric experiments, and the fluorescence spectra were recorded by a TOA (Tokyo, Japan) Flatbed Recorder FBR-251A.

2.2. Reagents

As a migrating buffer component, sodium tetraborate (borax; Wako, Osaka, Japan) was used. Nonionic surfactants, such as polyoxyethylene (23) lauryl ether (Brij-35, Wako), polyoxyethylene (20) cetyl ether (Brij-58, Wako), polyoxyethylene (20) stearyl ether (Brij-78, Aldrich, Milwaukee, WI, USA), were used as a modifier in the migrating solution.

As analyte anions, salts of sodium naphthalene-1-sulfonate (1-NS), sodium naphthalene-2-sulfonate (2-NS), disodium naphthalene-1,5-disulfonate (1,5-NDS), and disodium naphthalene-2,6-disulfonate (2,6-NDS) were used. Carboxylic acids, such as 1-naphthoic acid (1-NC), 2-naphthoic acid (2-NC), naphthalene-2,3-dicarboxylic acid (2,3-NDC), naphthalene-2,6-dicarboxylic acid (2,6-NDC), phthalic acid (PH), isophthalic acid (i-PH), and terephthalic acid (t-PH), were also used as analyte anions after being neutralized with equivalent or twice equivalent amounts of NaOH. The analytes were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Water used was a deionized and distilled one.

2.3. CZE measurement

A migrating solution containing 0.01 *M* borax (pH 9.2) and a certain amount of nonionic surfactants (0–3.5%, w/v) was filled both in a cathodic and an anodic reservoir, as well as into a capillary. A sample solution containing 11 kinds of analyte anions (1×10^{-5} *M* each) and 3% (v/v) ethanol (a marker of the electroosmotic flow) was introduced into the capillary from the anodic end by the vacuum system for 3 s (injection volume, about 9 nl). A voltage of 15 kV was then applied, and the analyte anions were detected photometrically on the cathodic end of the capillary at 230 nm. Throughout the measurement, the capillary was held in a constant temperature room at 35°C. The electrophoretic mobility of the analyte anions was calculated in a usual manner.

2.4. Fluorescence spectra measurement

A solution containing 1×10^{-5} *M* analyte anion, 0.01 *M* borax and a certain amount of nonionic surfactant (0–3.0%, w/v) was prepared, and the fluorescence spectrum was measured.

3. Results and discussion

3.1. Separability improvement of positional isomers by the use of nonionic surfactants

Anionic positional isomers have been separated by the use of cationic polyelectrolyte [16]and quaternary ammonium ions [17,18]. In this study, some nonionic surfactants were investigated as modifying reagents to separate such isomers by binding the analytes to the micelle. The typical electropherograms are shown in Fig. 1. In the absence of nonionic surfactants, the isomers were not separated as is shown in Fig. 1a. However, the separability of the isomers was improved by adding Brij-58 in the migrating solution, as is shown in Fig. 1b. Other nonionic surfactants, such as Brij-35 and Brij-78, also improved the separability among the isomers.

To obtain sufficient separation, nonionic surfactants added to the migrating solution at concentration levels of a few percent (several tens of m*M*) is required; the amount seems to be quite enormous. However, the electric current during electrophoresis was almost constant, about 7 μA, in spite of the change in the concentrations of the surfactants. Therefore, the MEKC separation using nonionic surfactants has the advantage that high concentrations of additive are allowed without an increase in electric current, as is noted in the precedent study [12].

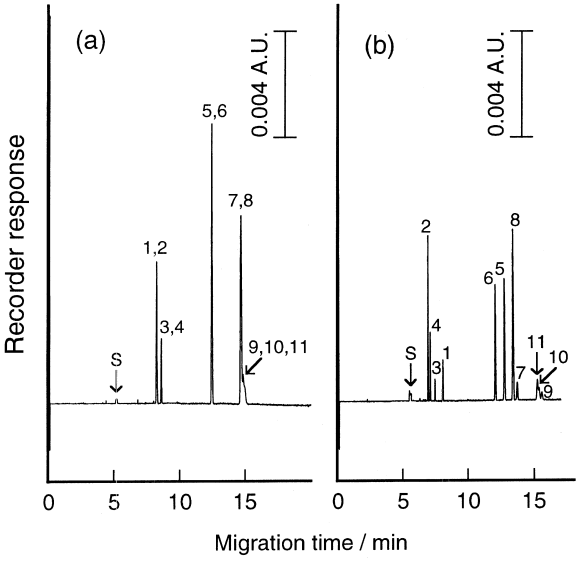

Fig. 1. Electropherograms for 11 kinds of anions in the absence and presence of nonionic surfactant. CE conditions: applied voltage, 15 kV; detection wavelength, 230 nm; capillary temperature, 35°C; injection period, 3 s. Migrating solution: (a) 10 m*M* borax; (b) 10 m*M* borax+26.7 m*M* (3.0%, w/v) Brij-58. Sample solution: 1×10^{-5} *M* 11 kinds of anions. Signal identifications: (1) 1-NC; (2) 2-NC; (3) 1-NS; (4) 2-NS; (5) 2,3-NDC; (6) 2,6-NDC; (7) 1,5-NDS; (8) 2,6-NDS; (9) PH; (10) i-PH; (11) t-PH. S, ethanol (EOF marker).

3.2. Electrophoretic mobility change with increasing concentrations of nonionic surfactants

The apparent electrophoretic mobility of the analyte anions, $-\mu'_{ep}$, was plotted against the concentration of the nonionic surfactants (Fig. 2). The mobility decreased with an increase in the concentration of the surfactants. It suggests that the analyte anions are likely to bind to the nonionic surfactant micelle and change in their apparent molecular mass and/or apparent molecular volume, even though the apparent charge does not change. The plots also indicate that the degrees of the apparent mobility decrease are different among the

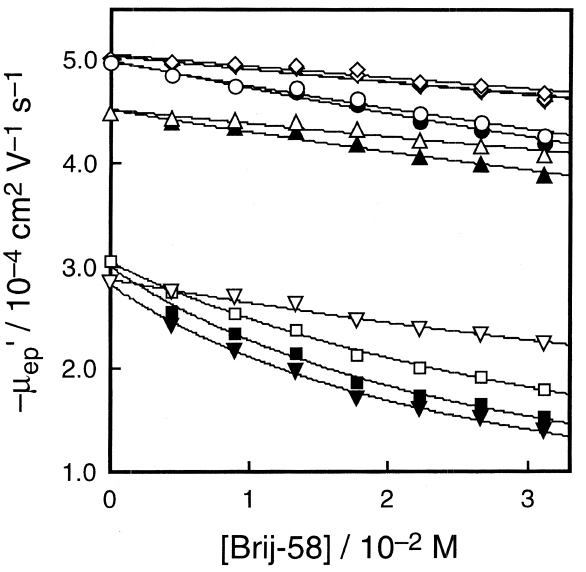

Fig. 2. Changes in electrophoretic mobility of anions with increasing concentrations of Brij-58. Conditions, except for nonionic surfactant concentrations, are the same as in Fig. 1. (▽) 1-NC; (▼) 2-NC; (□) 1-NS; (■) 2-NS; (△) 2,3-NDC; (▲) 2,6-NDC; (○) 1,5-NDS; (●) 2,6-NDS; (◇) PH; (♦) i-PH; (◆) t-PH.

isomers; therefore, prominent separation can be attained.

Considering the reactivity of each anion from the view point of charge, the degree of the decrease in the mobility is larger in monovalent anions than in divalent anions, which indicates that less charged ions are more reactive with the nonionic surfactant micelles. This is quite reasonable, because the nonionic surfactant micelles offer hydrophobic media and, therefore, more charged ions or more hydrophilic ions are more difficult to bind to such hydrophobic media.

3.3. Binding constants

The binding constants of analyte anions to the nonionic surfactant micelles were determined by analyzing the change in electrophoretic mobility. In the presence of sufficiently excess amounts of micelle compared to the analytes, an analyte anion, A^{n-} ($n=1, 2$), is assumed to be bound to the micelle by 1:1 as is written in reaction (1) and its equilibrium (Eq. (2)).

$$A^{n-} + (NIS)_m \overset{K_B}{\rightleftarrows} A^{n-} - (NIS)_m \quad (1)$$

$$K_B = \frac{[A^{n-} - (NIS)_m]}{[A^{n-}]\,[(NIS)_m]} \quad (2)$$

where $(NIS)_m$ denotes the micelle of nonionic surfactants, and K_B is a binding constant. The concentration of the micelles was calculated from the molar concentration of the surfactant and its aggregation number, A.N.; the A.N. values used are 40 for Brij-35 and 70 for Brij-58 [19]. In this study, the concentrations of the surfactants or the concentrations of the micelles examined are sufficiently larger than that of analyte anions; the concentration of micelles is about 10–50-fold that of the anions. Therefore, only a 1:1 reaction can be expected.

The apparent electrophoretic mobility of the analyte anions can be written as in Eq. (3) from the mass balance of A^{n-} and Eq. (2).

$$-\mu'_{ep} = \frac{1}{1 + K_B[(NIS)_m]} \cdot (-\mu_{ep}) + \frac{K_B[(NIS)_m]}{1 + K_B[(NIS)_m]} \cdot (-\mu_{ep,C}) \quad (3)$$

where $-\mu_{ep}$ is the electrophoretic mobility of a free anion and $-\mu_{ep,C}$ is that of anion bound to the micelle. When an analyte anion is bound to the micelle, the apparent molecular mass and/or the molecular volume of the anion increases significantly; therefore, the value of $-\mu_{ep,C}$ can be considered to be almost zero. A non-linear least-squares method based on the previous study [17,18]was applied to the calculation, where a series of the value, $-\mu'_{ep}$, and the concentration, $[(NIS)_m]$, was input and the values of K_B and $-\mu_{ep}$ were optimized. Obtained binding constants are summarized in Table 1. To compare the reactivity, the equilibrium constants based on the monomer concentration of the surfactant, $K_{B,(mon)}$, are also summarized. From the constants obtained in this work, it is noted that monovalent anions are more reactive than divalent ones, as is noted from the degree of the mobility decrease.

Table 1
Binding constants of anions to the nonionic surfactant micelles

Nonionic surfactant	Log K_B [a]										
	1-NC	2-NC	1-NS	2-NS	PH	i-PH	t-PH	1,5-NDS	2,6-NDS	2,3-NDC	2,6-NDC
Brij-35	2.70±0.15	3.22±0.05	3.06±0.07	3.20±0.04	2.19±0.21	2.24±0.19	2.27±0.21	2.50±0.10	2.56±0.10	2.31±0.19	2.51±0.13
	(1.09±0.14)	(1.61±0.04)	(1.45±0.06)	(1.60±0.04)	(0.59±0.22)	(0.63±0.19)	(0.67±0.22)	(0.90±0.09)	(0.96±0.10)	(0.71±0.19)	(0.90±0.14)
Brij-58	2.79±0.11	3.73±0.06	3.20±0.06	3.35±0.09	2.22±0.27	2.26±0.21	2.28±0.21	2.54±0.11	2.61±0.09	2.33±0.23	2.54±0.14
	(0.94±0.11)	(1.53±0.06)	(1.35±0.06)	(1.50±0.09)	(0.37±0.27)	(0.41±0.21)	(0.43±0.21)	(0.70±0.11)	(0.76±0.09)	(0.49±0.22)	(0.69±0.13)
Brij-78	(0.91±0.07)	(1.50±0.05)	(1.34±0.04)	(1.50±0.06)	(0.32±0.09)	(0.35±0.16)	(0.40±0.14)	(0.69±0.11)	(0.76±0.06)	(0.42±0.11)	(0.69±0.07)

[a] Error, 3σ; values in parentheses are log $K_{B,(mon)}$ values.

3.4. Comparison of the kinds of nonionic surfactants on the reactivity

The binding reactivity was examined from the view point of the hydrophobicity of the surfactants or surfactant micelles. It is understood from the structure of the surfactants that Brij-35 can belong to the most hydrophilic surfactant of the three and Brij-78 the most hydrophobic.

The mobility changes of the analyte anions, as well as the binding constants, were compared with each nonionic surfactant. The decreases in the mobility are shown in Fig. 3, where 2-NC and 2,3-NDC are adopted as examples. The decreases are almost identical when the kinds of the surfactants are varied. Similar decreases were found in other analyte anions. Comparing the binding constants of Brij-35 with those of Brij-58, the values are found to be almost identical. Therefore, the nonionic surfactant micelles examined in this work possess similar binding property for analyte ions, which suggests that the analyte anions should be bound to the polyoxyethylene moiety of the micelles. The similar binding property also suggests that the hydrophobicity of the polyoxyethylene moiety in the three nonionic surfactants examined is considered to be almost the same.

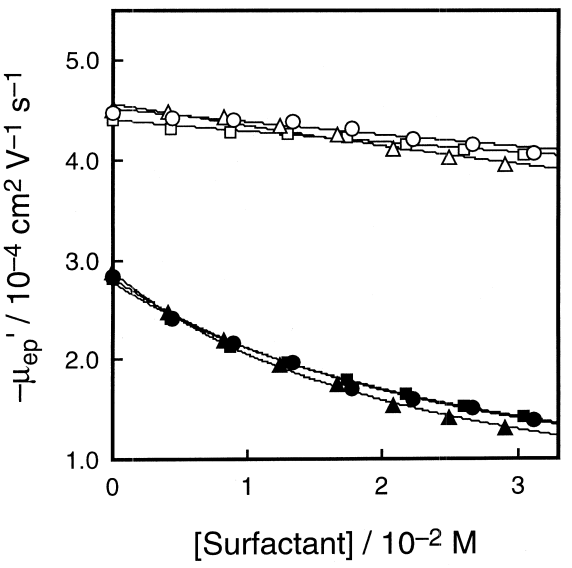

Fig. 3. Changes in electrophoretic mobility of 2-NC and 2,3-NDC with the kind of surfactant. Conditions, except for nonionic surfactant concentrations, are the same as in Fig. 1. Open symbols, 2,3-NDC; closed symbols, 2-NC. Nonionic surfactants: (△ and ▲) Brij-35; (○ and ●) Brij-58; (□ and ■), Brij-78.

3.5. Comparison of the reactivity with ion associability

The differences in the reactivity examined among the anionic isomers were compared from the mobility change, as well as the binding constants. The reactivity among the isomers is in the orders: 2-NS > 1-NS, 2-NC > 1-NC, 2,6-NDC > 2,3-NDC, 2,6-NDS > 1,5-NDS, and t-PH > i-PH > PH. Such orders are the same as in ion association-capillary zone electrophoresis using tetrabutylammonium ion as a pairing cation [17]. These results also show that the analyte anions are bound to the nonionic surfactant micelles by hydrophobic interactions. However, the binding constants are quite larger than the ion association constants, although the binding reaction does not include inter-ionic interaction. This can be understood from the fact that the ion association reaction in an aqueous solution is weaker than the distribution reaction of the ions in the hydrophobic media [17].

3.6. Fluorescence intensity measurement for the analysis of the reaction

The reactivity of anions with the surfactants was also investigated by the change in fluorescence intensity. The fluorescence spectra for 2-NC, as a more reactive example, and 2,3-NDC, as a less reactive one, are shown in Fig. 4. Emission around 360 nm is attributed to the fluorescence of the aromatic anions, when being excited at 285 nm. The fluorescence intensity for 2-NC significantly decreased with increasing concentrations of Brij-58. On the other hand, the fluorescence intensity for 2,3-NDC was almost identical even in the presence of Brij-58 at higher concentrations. The changes both in the mobility and the intensity are compared with each other, as shown in Fig. 5. The ratio of the decrease is almost similar to that obtained by the

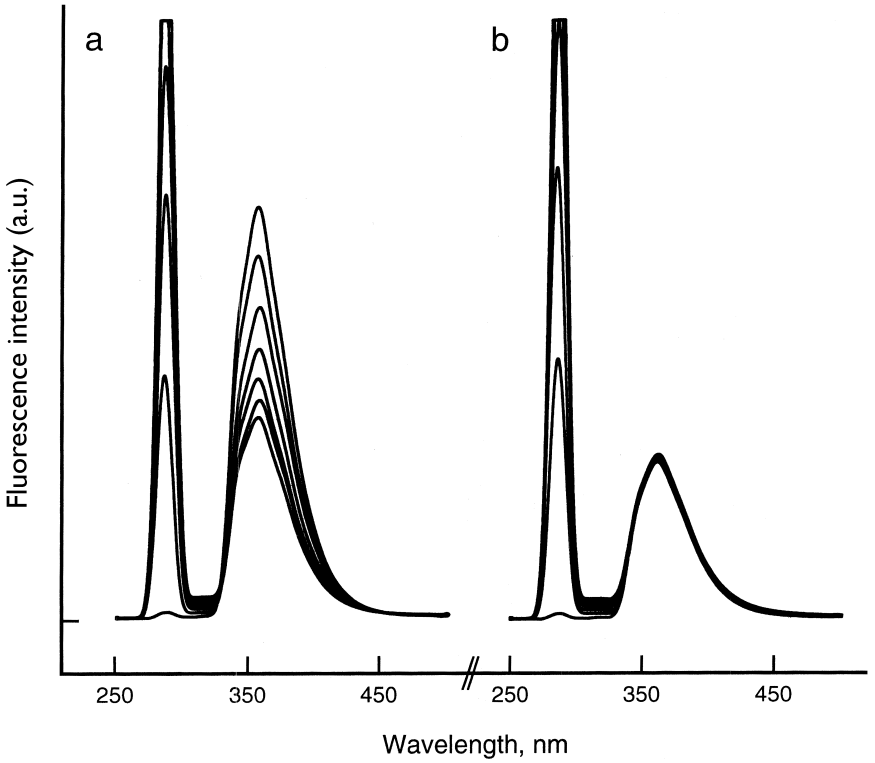

Fig. 4. Fluorescence spectra of 2-NC and 2,3-NDC in the absence and in the presence of Brij-58. Excitation wavelength, 285 nm; solution, 10 m*M* borax+0.0–26.7 m*M* Brij-58+1×10^{-5} *M* aromatic anion. Aromatic anions: (a) 2-NC; (b) 2,3-NDC. Concentrations of Brij-58: from top to bottom, 0.0, 4.4, 8.9, 13.4, 17.8, 22.2 and 26.7 m*M*. Peaks around 285 nm are attributed to Rayleigh scattering enhanced by the presence of nonionic surfactant micelles.

mobility measurement, which indicates that the reaction can be analyzed both by the mobility measurement and by the fluorescence measurement. It also indicates that the analysis by the mobility measurement is quite valid.

Any wavelength shift of the emission spectra was not observed with the anions in the presence of Brij-58, as is shown in Fig. 4, which indicates that the anions are not incorporated in the hydrophobic core of the surfactant micelles. The results also suggest an important role for the polyoxyethylene moiety of the nonionic surfactant micelles.

4. Conclusion

In this study, an analysis method for the reaction between ionic substance with nonionic surfactant micelles was proposed, where the mobility change in capillary zone electrophoresis was used. Aromatic anions possessing small charges are proved to be more reactive with the micelles through hydrophobic interactions. Capillary electrophoresis with nonionic surfactant micelles is also found to be very useful for the separability improvement in electrokinetic chromatography.

Acknowledgements

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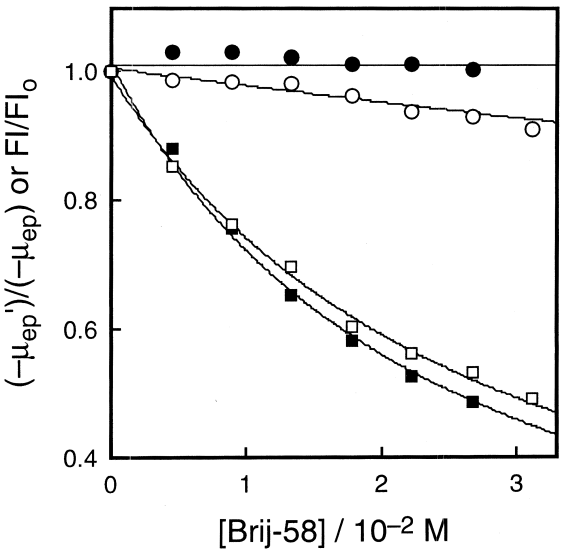

Fig. 5. Decreases in the electrophoretic mobility and the fluorescence intensity with increasing concentrations of Brij-58. The mobility and the intensity are standardized with the initial ones, $(-\mu_{ep})$ and FI_o; they are presented as $(-\mu'_{ep})/(-\mu_{ep})$ and FI/FI_o, respectively. Conditions, except for nonionic surfactant concentrations, are the same as in Fig. 1. Open symbols, results of mobility; closed symbols, results of fluorescence intensity. Analyte anion: (□ and ■) 2-NC; (○ and ●) 2,3-NDC.

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