The Isoelectric Point on Ethyleneoxide Adducts of Amino Acid

Part II

by *Tadashi ISHIKAWA** & *Hisao HIDAKA***

Summary

The ethyleneoxide adducts of α-alanine, β-alanine, aspartic acid or monosodium glutamate were synthesized respectively. The prepared adduct was sodium salt of N-etho amino acid, where the number of the etho groups was one, two and three by the identification of NMR and paper-chromatography. The electric conductivity to pH was measured with Kohlrash bridge and the following results were obtained.

(1) The isoelectric point of all adducts had the broad-spread zone in the range of pH 3 to 12.

(2) The broadness of isoelectric zone was larger as the increasing of the added ethyleneoxide (EO) mol-number with N-atom in the case of α-alanine, monosodium glutamate and aspartic acid as well as glycine.

(3) The isoelectric point of EO adducts was shifted to acid side. From this fact, it was found that the basicity of N-atom was weaker by the N-alkyltion of EO addition.

Introduction

Various amino acid have been employed as commercial sources in the fields of chemical industries.[2] Recently various derivatives of amino acid are used for a surfactant, a chelating agent, a synthesized polymer and fine chemical reagents. It is well-known that their derivatives have excellent properties of no toxicity and biodecomposition after usages.[3] As amino acid possesses the active hydrogen functional groups having both amino and carboxylic group, EO is added with both groups.[4]

Although the EO adduct on the case of lauryl ester of lysine was reported,[5] there are few papers on the addition reaction of EO only with N-atom of amino acid.[6,7] The isoelectric point of alkyl betaine as one of amphoteric surfactants was reported by Komori *et. al.*[8] In our previous paper, EO adducts of glycine and their isoelectric zone have already published.[9] The subject of this studies is the basic experimental to prepare an amphoteric surfactant having just pI 7.00

* 理工学部化学科教授　物理化学
** 理工学部化学科助教授　物理化学

isoelectric point. The EO addition reaction of α-alanine, β-alnaine, aspartic acid, and monosodium glutamate and its isoelectric point were investigated in this paper.

Experimental

Materials; Each amino acid was employed with recrystalization. DL-α-alanine; (Wako Pure Chem, Ind. Co., Ltd,) β-alanine; (Tokyo Kasei Ind. Co., Ltd.) DL-aspartic acid and Monosodium glutamate; (Wako Pure Ind. Co., Ltd.)
Ethyleneoxide; EO was a commercial origine by Nisho-Yuka Chem., Ind. Co. Ltd. and used without purification directly from the container.
NMR spectra; MH Spectrometer (100 MH_z) by Japan Electric Optical Laboratory Co., Ltd. was used in D_2O solvent on the base of $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$ (DSS) as an internal reference.
Paper-Chromatography; The presence of unchanged amino acid and various EO adducts was checked with paper-chromatography on the same condition as described in our previous paper in detail.[9] Each reaction mixture was developed at the same time in a large developing vessel without the difference of experimental condition.
The measurement of Electric Conductivity; Kohlrash Bridge K-IA made by Yokokawa Electric Works was employed. Two grams of a purified sample was weighted to solve 100 ml of aqueous solution. Its solution was devided into two parts for acid and alkali side. The conductivity was measured at 30°C in the thermostat similarly as reported in previous paper.[9]
Preparation of N-etho amino acid

Each amino acid (0.4 mol) was solved in 20% aqueous solution of 0.4 mol sodium hydroxide. Its solution was placed in 500 ml four necked round bottle flask equipped with a stirrer, a dropping funnel and a condenser. 35.2g (0.8mol-2 fold mol equivalent) of EO or 105.6g (2.4mol-6 fold mol equivalent) of EO was added dropwise from funnel during stirring in an icecooling bath for an additional 1 hr, the heating at 80~90°C was allowed to continue stirring for another period of 4 hr.

Next, the water in the reaction mixture was evaporated to obtain high viscous yellowish liquid. The ethyleneglycol which was by-produced by the reaction of EO with H_2O was removed off by the acetone-topping.
The unsoluble matter in acetone was solved in MeOH and sodium chloride crystal was filtered off. Then, the solution was rearranged in the range from pH 6.0~6.5 by HCl-MeOH solution. After removing MeOH, the residue was solved in EtOH. The soluble matter in EtOH was separated and EtOH was evaporated off. The residual oily liquid was dried *in vacuo*.
In the case of the EO adduct of aspartic acid, it was so insoluble with H_2O, MeOH, and EtOH that sodium chloride could not be removed.
So if was used as a sample with purification. The preparations and purification were in further detail reported in our previous paper.[9]

Results and Discussion

When EO was introduced to the flask containing each amino acid and alkali solution at the ratio of 2-fold mole equivalent, various EO adducts were obtained and the Rf values of paper-chlomatography are shown in Fig 1.

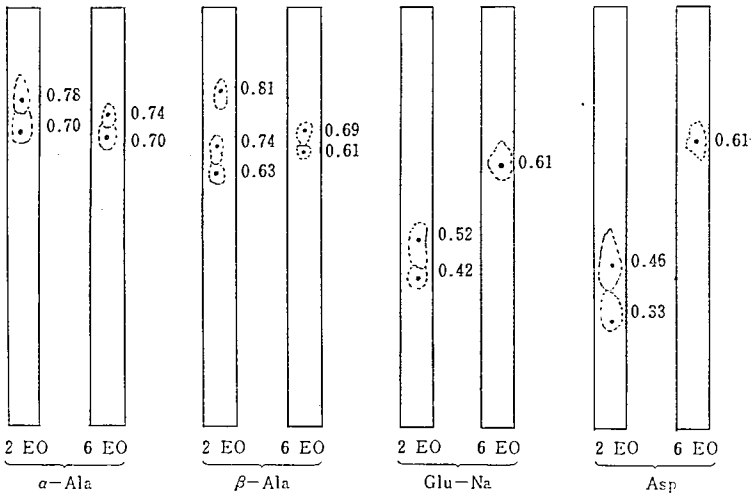


Fig. 1 Paper-Chromatography of adducts

In the case of α-Ala+2 EO, the unchanged α-Ala are present at Rf 0.70 and α-(2-hydroxyethyl) alanine of the 1 : 1 adduct at Rf 0.74, while in the case of α-Ala+6 EO 1 : 2 adduct or 1 : 3 adduct existed.

β-Ala+2EO was given the unchanged β-Ala (Rf 0.63), the 1 : 1 adduct (Rf 0.74), and the 1 : 2 adduct (Rf 0.81). On the other hand β-Ala+6EO was given the 1 : 3 adduct (Rf 0.69) and the unchanged β-Ala (Rf 0.61).

There were the unchanged Asp (Rf 0.33) and the 1 : 1 adduct (Rf 0.46) in the case of Asp+2EO, and the 1 : 3 adduct in the case of Asp+6EO.

Glu-Na+2EO was given the unchanged Glu-Na (Rf 0.42) and the 1 : 1 adduct (Rf 0.52), while Glu-Na+6EO is given the 1 : 3 adduct.

NMR spectra of N-hydroxyethyl α-alanine, namely the 1 : 1 adduct is shown in Fig 2-1. Although high resolution NMR Spectra contain peaks which are difficult to assign to any position of proton known to be present, each chemical shift position is summerized in each figure below. Methyl proton of α-alanine gave 1.50 ppm shift value at doublet peak. Protons (c) and (d) of the hydroxyethyl group lie at 3.94 ppm and 3.35 ppm in the form of triplet. Fig 2-2 is NMR spectra of the 1 : 3 adduct of aspartic acid. Each chemical shift value and structural formula were written. NMR spectra of N-hydroxyethoxy ethyl glutamaic acid is shown in Fig 2-3. The structure was determined by proton shift and integral value. However no spin-spin coupling constants were counted in all cases. NMR spectra were measured on each amino acid adduct of EO; 1 : 1 adduct, 1 : 2 adduct and

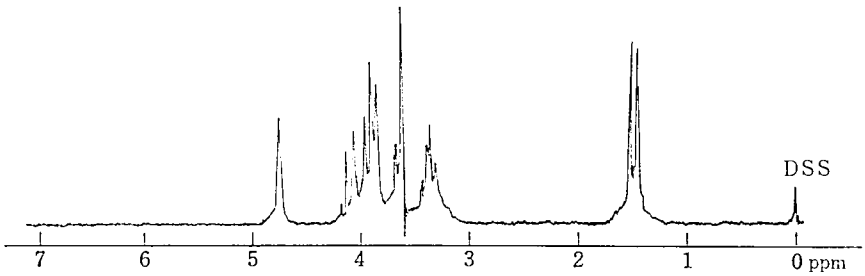
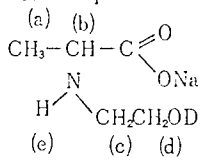


Fig. 2-1 NMR spectra of N-hydroxyethyl alanine



assignments

(a) 1.50 (doublet) (e) 3.64 (singlet)
(b) 4.12 (quartet) (f) 4.78 HDO
(c) 3.94 (triplet)
(d) 3.35 (triplet)

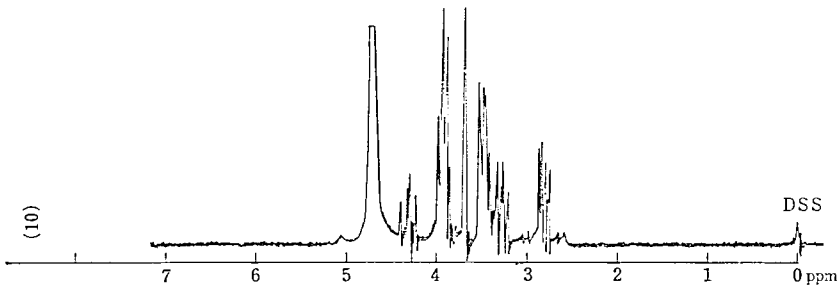
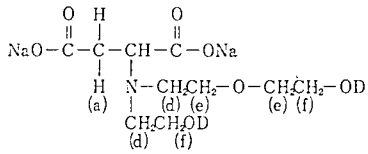


Fig. 2-2 NMR spectra of N-hydroxyethyl N-hydroxyethoxyethyl aspartic acid



assignments

(a) 2.68 (doublet)
(b) 2.86 (doublet)
(c) 4.38 (triplet)
(d) 3.94
(e) 3.26
(f) 3.53
(g) 4.70 HDO

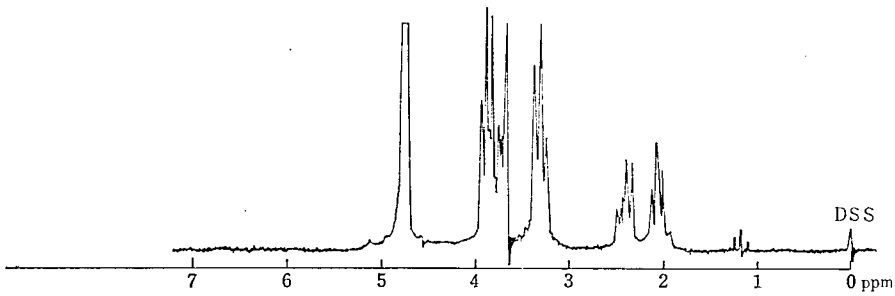
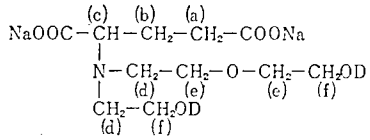


Fig. 2-3 NMR spectra of N-hydroxyethyl N-hydroxyethoxyethyl glutamic acid



assignments

(a) 2.08
(b) 2.36
(c) 3.64
(d) 3.89
(e) 3.18
(f) 3.34
(g) 4.70 HDO

1 : 3 adduct. But these charts were omitted.

The isoelectric point was measured by the electric conductivities to the pH variation. The results of α-Ala+EO adducts are shown in Fig 3. On the bases

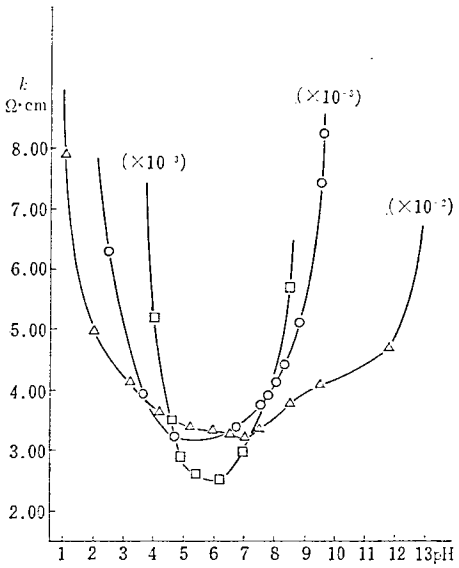


Fig. 3
Isoelectric point of α-alanine-ethyleneoxide adducts
□ Pure α-Alanine 100g/l+NaCl 0.07g
Literature value pI=6.09
○ Sodium α-Alanine+2EO
△ Sodium α-Alanine+6EO

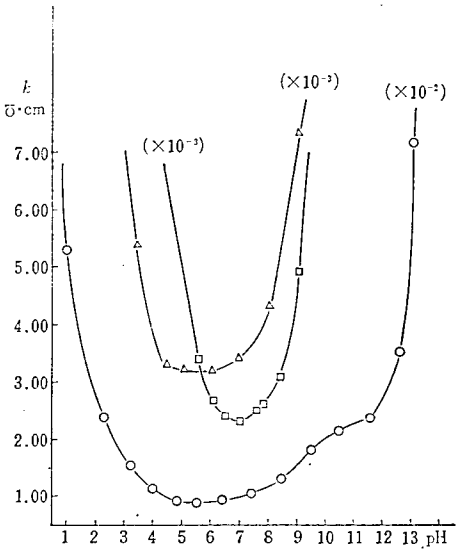


Fig. 4
Isoelectric point of β-alanine-ethyleneoxide adduct
□ Pure β-Alanine 100g/l+0.14gNaCl pI=6.90
○ Sodium β-Alanine+2EO
△ Sodium β-Alanine+6EO

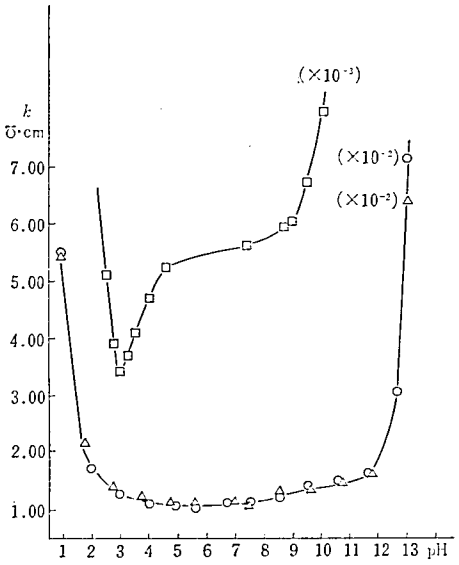


Fig. 5
Isoelectric of aspartic acid-ethyleneoxide adduct
□ Pure Aspartic acid 5g/l+0.14gNaCl
Ltierature value pI=2.77
○ Sodium Aspartic Acid+2EO
△ Sodium Aspartic Acid+6EO

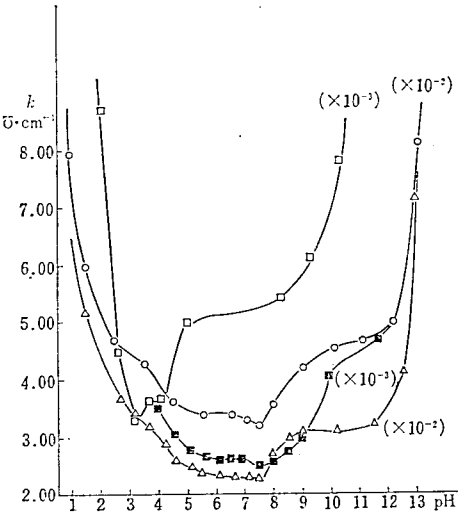


Fig. 6
Isoelectiric point of glutamic acid-ethyleneoxide adduct
□ Pure Glutamic acid 5.8g/l+1.4gNaCl
Literature value pI=3.22
○ Sodium Glutamate+2EO
△ Sodium Glutamate+6EO
■ Pure mono Sodium Glutamate

of the isoelectric point, the point of pure α-Ala was measured and was in agreement with the literature date (pI=6.00). The point of α-Ala+2EO is shifted to the acid side of pI=5.3, while the point of α-Ala+6EO had the broad-spread zone in all range of pH.

The point of β-alanine adducts is shown in Fig 4.

Pure β-Ala had pI=6.90. However it is characteristic that β-Ala+2EO showed broad isoelectric range in comparison with β-Ala+6EO.

β-Ala+6EO possesed the isoelectric zone at pI=5.0~6.0 and its shifted to the acid side rather than of pure β-Ala.

Fig 5 is the result of isoelectric point on aspartic acid. Its point of pure aspartic acid existed at pI=2.77 owing to two amino groups and one carboxylic group. Both Asp+2EO and Asp+6EO indicated the same curves and had the same conductivity in the range of pH 3 to 12.

The glutamic acid and its derivatives are shown in Fig 6.

Pure glutamic acid had pI=3.22 and was agreed with data of literature.

The point of mono sodium glutamate was measured as a reference too.

Glu+6EO indicated broad isoelectric zone than Glu+2EO. As the N-alkylation was carried out by EO, the range of isoelectric point became wider.

Further, the adducts having a hydroxyethyl group or an etho group decreased the solubility, so the turbidity or precipitation did not arise even in all pH range.

Acknowledgments

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