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By

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Amino acids, having the general formula $RCH(NH^+_3)COO,^-$ are qualified for surface active substances, since each amino acid has both hydrophyilic ($^-COO^-$, and $-NH^+_3$, dipol ions) and Lipotropic radicals (-R).

In living organisms, there exist several interfaces of oil-water, water-solid etc., where amino acids and proteins play many physiologically and chemically interesting rôles. Adsorption of amino acids and formation of insoluble protein films at the interfaces are considered very important factors for protein synthesis in vivo[1]. Recently, based on the studies of the phenomena which occured at such interfaces, the investigations of the energy of protein synthesis, amino acid orientation in protein molecules, protein specificities and other problems, have been attempted by several workers[2][3].

This paper reports the results of the measurements of surface potential of amino acid solution in air-water interface to study the amino acid adsorption at the surface where protein synthesis is postulated to take place.

Methods and Materials

Apparatus : In this experiment, an apparatus of Zisman-type[4] modified by Koga was used.[5] The arrangement is shown in Fig 1. To avoid electrical disturbances, the whole instrument is enclosed in metallic cage.

A reference electrode A faces the specimen B under meaurement. The electrode A is vibrated up and down by a dynamic speaker driving unit. This results in cyclic change of the charging and discharging current through the capacitance. This signal is detected through an amplifier. The compensating voltage, indicated by a voltmeter V, is applied to cancel this signal.

Measurement of surface potential : A Petri-dish of 3.0 cm diameter set on a holder E, 2 cc of redistilled water is poured into the dish. After making the reference electrode A vibrate, the water surface is brought up to the electrode
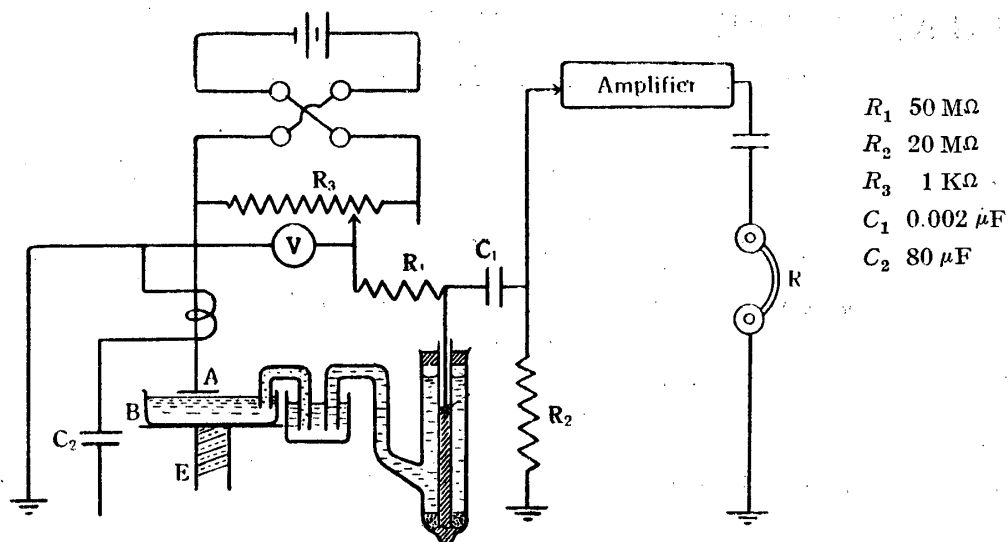
R_1 50 MΩ
R_2 20 MΩ
R_3 1 KΩ
C_1 0.002 μF
C_2 80 μF

Fig. 1.

A as nearly as possible. The amplifier is switched on. Hearing the sound by receivers *R*, the voltmeter is read when the sound is at the lowest. The reading is expressed as V water.

A test solution of amino acid is then poured into the Petri-dish *B* by a capillary pipet, and the potential is measured as described above. If V-sol. is the reading, the surface potential of amino acid solution is given by $\varDelta V = $ Vwater − Vsol.

To obtain a good result, it is desirable to bring the dish *B* towards the electrode *A* as near as possible. The result is reproducible within ± 5 mV.

Cleaning of Petridish : All glasswares must be cleaned thoroughly. First, the wares were washed with soap solution and then immersed in potassium bichromate-sulfuric acid soln. ; washed with tap water; next with distilled water two or three times, and dried over filter paper letting them lean against a glass rod. Thoroughout the cleaning, a hooked glass rod was used to keep them clean.

Distilled water : Redistilled water, treated with acidic potassium permanganate solution, then with NaOH solution and again distilled was used for the cleaning.

Amino acids : Amino acids were obtained commercially or prepared according to the usual methods. Each amino acid was recrystallized several times from water or ethyl alcohol. Specific rotations of these L-amino acids used are as follows.

L-phenylalanine	$[\alpha]_D^9$	− 35°
L-leucine	$[\alpha]_D^9$	10.3°
L-tyrosine	$[\alpha]_D^9$	−8.1°

Results

Surface potential of aliphatic mono-amino mono-carboxylic acids : Surface potential of the solution of the aliphatic amino acid is shown in Table 1. From the data given in the table it is found that ΔV increases with increasing methylen group in length. The DL-leucine is remarkably large.

Table 1. Surface potential of aliphatic amino acids solution.

Substance	Concentration (mol.)	Surface potential ΔV (mV)
Glycine	0.1	10
DL-Alanine	0.1	80
DL-α-Amino n-butyric acid	0.1	80
DL-α-Amino n-valeric acip	0.1	90
DL-Leucine	0.1	120

Difference of surface potential of L-amino acids and DL-amino acids : In a living organism, a selective utilization of L-amino acids is generally observed. Thus, the effect of the L-amino acids on the surface was compared with that of the DL-amino acids. The results are shown in Table 2. The surface potential of each L-amino acid solution was higher than that of the corresponding DL-amino acid at the same concentration. Since water solubilities of tyrosine and phenylalanine are much smaller than that of leucine, the surface potential measurement of a more concentrated solution was impossible.

Table 2. Surface potential of DL- and L-amino acids solution.

Substance	Concentration (mol.)	Surfaea potential ΔV (mV)	
		L-form	DL-form
Phenylalanine	0.014	20	20
	0.021	40	30
	0.033	70	40
	0.035	80	60
	0.041	140	120
Leucine	0.026	20	0~10
	0.05	50	30
	0.08	80	60
Tyrosine	0.0017	40	20

Surface potential of acidic and basic amino acids solution : These amino acids have more hydrophylic groups and show a characteristic action from the physiological standpoint. Table 3 shows the data obtained.

The surface potential of L-aspartic acid and L-lysine were found to be nearly

Table 3. Surface potential of acidic and basic amino acids solution.

Substance	Concentration (mol.)	Surface potential ΔV (mV)
L-Glutamicacid	0.01	20
	0.02	60
	0.03	80
L-Aspartic acid	0.01	0~10
	0.02	10
	0.028	20
L-Arginine	0.01	20
	0.02	40
	0.03	40
	0.05	50
	0.1	70
L-Lysine	0.01	—
	0.02	0~10
	0.05	0~10

zero, while those of L-glutamic acid and L-arginine are remarkably high compared with the values of L-aspartic acid and L-lysine.

Surface potential of mixed solutions of acidic and basic amino acids: Surface potential of the mixed solution of L-glutamic acid plus L-lysine and L-glutamic acid plus L-arginine solutions was measured respectively. The results are shown in table 4.

Table 4. Surface potential of amino acids mixed solution.

Substance (equal mixture)	Concentration of amino acid respective (mol.)	Surface potential ΔV (mV)
L-Glutamic acid+	0.005	120
	0.01	150
L-Arginine	0.02	200
	0.027	240
L-Glutamic acid+	0.005	70
	0.01	100
L-Lysine	0.02	120

Table 5. Surface potential of dipeptides.

Substance	Concentration (mol)	Surface potential ΔV (mV)
Glycyl-glycine	0.1	40
Glycyl-leucine	0.02	40
	0.04	60
	0.05	70
	0.07	90
Glycine	0.1	20~0

It should be pointed out that the surface potentials of the mixed solution of amino acids are higher than the sums of individual amino acids.

Surface potential of peptides solutions : In the experiments glycyl-glycine and glycyl-leucine were used. Table 5 indicates that both of them show slightly higher values than those of glycine or leucine at the same concentration.

Discussion

In general, the surface potentials of monoamino monocarboxylic acid solutions increase as the hydrophobic radicals increase in length. This agrees with the results of the surface tension measurements[6][7] and indicates that the quantity of amino acids adsorbed to the surface become greater with increasing surface potentials. It is very interesting that valine, leucine, isoleucine and phenylalanine, all of which have large R-radicals in their structure and show comparatively great adsorption to the surface, are essential for the mammlian body. The fact that the surface activity of L-amino acids are greater than those of DL-amino acids in the cases of leucine, phenylalanine and tyrosine, is supported by the surface tension measurements[8]. Such a result might aid in understanding the functional rôle of the surface in protein synthesis. In case of acidic and basic amino acids, there might be expected a lower surface activity due to their moleuclar structure. According to the surface tension measurements, it is indicated that L-aspartic and L-glutamic acids show slightly negative adsorption, L-arginine also shows slightly negative but only when the concentration of L-arginine is high. While, the surface potential values of aspartic acid and lysine are almost zero, on the contrary those of glutamic acid and arginine are considerable large.

The mixed solution of these acidic and basic amino acids show greater values than the individual solution. This might be due to the attractive interaction of each amino acid molecule. Further discussion on this point will be reserved for another report.

Summary

The surface potential measurements of aqueous solutions of such amino acids as glycine DL-alanine, DL-α-amino-n-butylic acid, DL-valine, DL-leucine, L-leucine, DL-tyrosine, L-tyrosine, DL-phenylalanine, L-phenylalainne, L-glutamic acid, L-aspartic acid, L-lysine and L-arginine have given the following results :

1. The surface potential values increase, in the series of mono-amino monocarboxylic acids, as the R-radicals of their molecules increase in lenght.

2. L-amino acids gave greater values than those of DL-form amino acids, in the cases of leucine, phenylalanine and tyrosine.

3. Both acidic and basic amino acids surface potentials were measured. The

values of L-aspartic acid and L-lysine were quite small (nearly zero), while those of L-glutamic acid and L-arginine were considerably large.

4. A much larger value was given in cases of mixed solutions of acidic and basic amino acids than the sums of the values which individual amino acid solutions gave.

5. As for glycyl-glycine and glycyl-leucine, it was found that both peptides gave slightly larger values than those of glycine or leucine both at the same concentration.

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