FORMATION AND CATALYTIC ACTIVITY OF HIGH MOLECULAR WEIGHT SOLUBLE POLYMERS PRODUCED BY HEATING AMINO ACIDS IN A MODIFIED SEA MEDIUM

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Abstract. Eighteen protein amino acids with milk casein composition were heated in a modified sea medium.

Marigranules were formed in the precipitates and soluble polymers were formed in the supernatant.

Time course of the reaction (ultraviolet spectra, the concentration of metal ions, and the concentration of amino acids in the supernatant) were measured. The time course of the formation of the soluble polymers was also studied by Bio-Gel P-2 column.

High molecular weight soluble polymers (HMWSP) were separated from low molecular weight ones by dialysis. It was shown that these polymers catalyzed the dehydrogenation of NADH. These polymers also catalyzed the coupled reaction between dehydrogenation of NADH and reduction of resazurin. This coupled reaction was accelerated by the light.

1. Introduction

Based on the consideration of the relationships of the composition of metal ions between the present sea and the present enzyme systems, Egami proposed the hypothetical reaction condition of primitive sea water (Egami, 1974). It has been called 'a modified sea medium', in which the concentration of six transition metal ions were 10^3 or 10^4 times greater and that of sodium chloride was less than those of the present sea. Egami and his coworkers have been studying the formation of biomolecules and oriented granules (Marigranules) in the modified sea medium (Hatanaka and Egami, 1977; Yanagawa and Egami, 1977; Okihana, 1979).

It is now generally accepted that small biomolecules such as amino acids, bases, sugars, and lipids had formed on the primitive Earth (Calvin, 1969; Ponnamperuma, 1972). They accumulated in the primitive sea, where the first life probably originated (Sagan, 1961).

Based on these two assumptions, namely (1) the modified sea medium has the same characteristics as the primitive sea and (2) the presence of amino acids in the primitive sea, we have been studying the formation and characteristics of soluble polymers (Okihana and Egami, 1979). This paper presents the time course of the thermal reaction of eighteen amino acids in the modified sea medium and the catalytic activities of the high molecular weight soluble polymers (HMWSP) formed by the thermal reaction.

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2. Materials and Methods

2.1. REACTION MIXTURE

Modified sea medium was composed of 10mM each of $MgSO_4$, $CaCl_2$ and K_2HPO_4, and 0.1 mM each of $Fe(NO_3)_3$, Na_2MoO_4, $ZnCl_2$, $Cu(NO_3)_2$, $CoCl_2$ and $MnCl_2$, and 1 wt% of amino acid mixture. The value of pH was adjusted to 5.2 by addition of NaOH. The solution was sterilized by filtration through 0.2 μm millipore filter membrane (Polycarbonate membrane, d = 47 mm, Nucleopore Corp.). Air was substituted by nitrogen gas.

2.2. AMINO ACIDS MIXTURE

Eighteen amino acids (generally occurring protein amino acids except asparagine and glutamine) were mixed in a ratio of milk casein: Ala 2.7, Arg 3.6, Asp 5.6, $(Cys\text{-})_2$ 0.3, Glu 20.5, Gly 1.8, His 2.6, Ile 5.7, Leu 8.8, Lys 7.1, Met 2.9, Phe 5.0, Pro 10.7, Ser 5.5, Thr 3.9, Trp 1.3, Tyr 5.5 and Val 6.4 wt%.

2.3. REACTION CONDITIONS

Two liters of the reaction mixture contained in a glass vessel with reflux was given an oil bath at 105° C. The end of reflux was sealed with mercury. An aliquot of the reaction mixture was taken out through a side cock at intervals for analysis.

The reaction mixture was heated for 6 weeks at 105° C. Hydrolysis was done with 6 N HCl at 110° C for 24 hr. Amino acid analysis was carried out by Durrum Model D–500.

The concentrations of metal ions were measured by Varian AA–175 Atomic Absorption Spectrophotometer.

Ultraviolet and visible absorption spectra were taken by Cary Spectrophotometer Model 17 and Gilford Model 2400–2.

2.4. FRACTIONATION OF POLYMERS

An aliquot of the samples were charged on Bio–Gel P–2 column (d = 1.2 cm, l = 50 cm). They were eluted by 0.01 M NH_4HCO_3 (pH = 8.0 ± 0.5) with the flow rate of 8 ml/h. Absorbances of the elutants at 275 nm were measured by Cary Spectrophotometer Model 17.

2.5. SEPARATION OF HMWSP

The reaction was stopped after 6 weeks of heating. After cooling the mixture, the soluble fraction was separated from the precipitates by low-speed centrifuge (3000 rpm, 10 min) at 4° C. The supernatant was dialyzed against 100 times volumes of the distilled water. After dialyzing four times, the undialyzable fraction was lypholyzed and was used as high molecular weight soluble polymers (HMWSP).

2.6. DEHYDROGENATION AND REDUCTION REACTIONS

The dehydrogenation of NADH or coupled reaction between NADH and resazurin were carried out in 3 ml quartz cells and absoptions at 340 nm or those at 340 nm and 600 nm

were measured. Each of the experiments was composed of four cells: (1) with buffer only as a blank to test instrumental error, (2) with NADH solution only, (3) with NADH and bovine serum albumin and (4) with NADH and HMWSP. Each cell was sealed and fixed in the cell holder during the measurements. Absorbances were automatically measured by Gilford Spectrophotometer Model 2400–2. To detect small changes in the high absoption range, both zero control in the console and recorder offset in the sample handling system were used. Each of the experimental conditions is described in the corresponding figure caption. To avoid contamination, one drop of chloroform was added to the system.

3. Results and Discussion

3.1. TIME COURSE OF REACTION

After heating for 0 hour (h), 8 h, 24 h, 1 week (w) 2 w, 3 w, 4 w and 5 w, an aliquot of the reaction mixture was removed by suction through the side cock of the reaction vessel.

The solution was clear before heating. After heating for 1 week, precipitates began to appear, and were composed of sheet-shaped and sphere-shaped Marigranules. The amount of Marigranules was linearly proportional to the reaction time (Okihana, 1979).

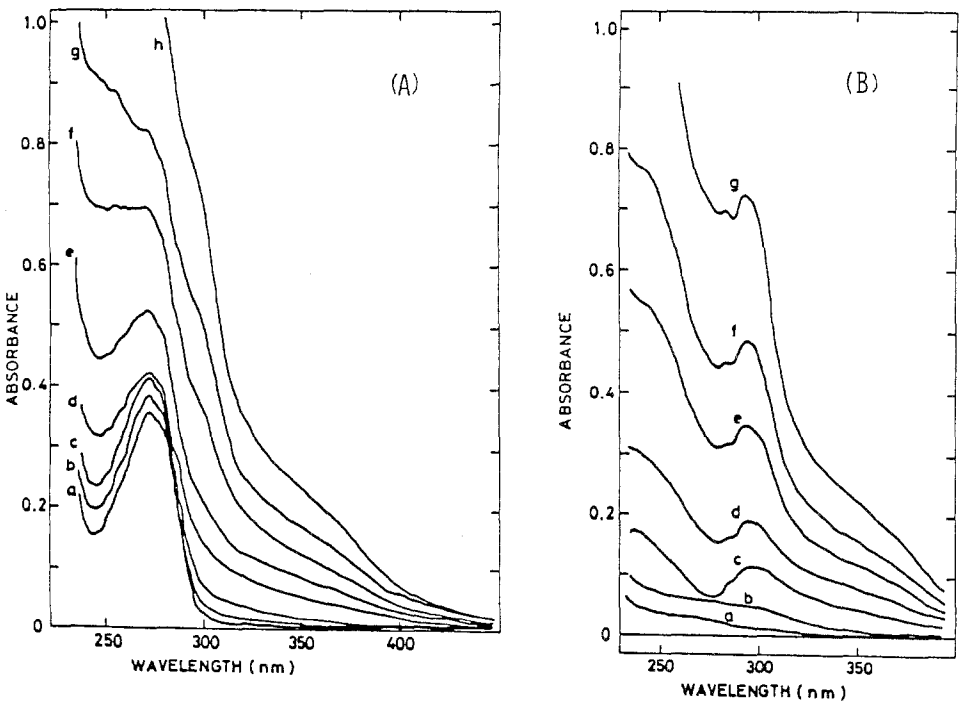

Fig. 1. A. The time course of the UV-spectrum and Vis-spectrum of the reaction mixture after heating for (a) 0 h, (b) 8 h, (c) 24 h, (d) 1 w, (e) 2 w, (f) 3 w, (g) 4 w and (h) 5 w. B. The difference in spectra of the UV-spectrum between the original mixture (0 h) and those after heating for (a) 8 h, (b) 24 h, (c) 1 w, (d) 2 w, (e) 3 w, (f) 4 w and (g) 5 w.

The reaction mixture changed its color by heating from transparent to dark brown via yellowish color. The original solution had an absorption maximum near 280 nm due to Trp, Tyr and Phe. This UV-spectrum gradually changed to a gently sloped one (Figure 1A). The difference spectra was calculated by comparing each new spectrum with the original (0 h), and had a maximum near 295 nm and a minimum near 280 mm (Figure 1 B). This change was mainly due to the decrease in the concentration of tyrosine and tryptophan in the supernatant (Table I(a).

Concentrations of each of the metal ions in the supernatant were measured by using atomic absorption methods. The potassium ion was most abundant and had an almost constant value during the reaction (20 mM). Concentrations of the other metal ions were recalculated with reference to the potassium ion and results are shown in Figure 2. Among

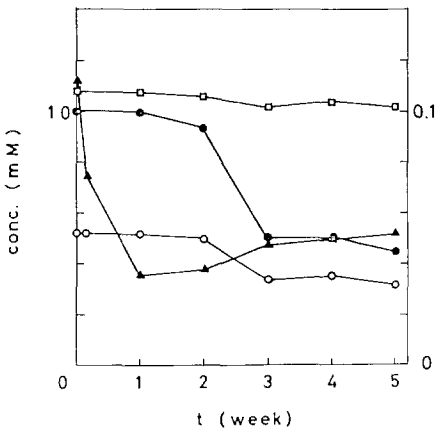

Fig. 2. The time course of each of the metal ions in the supernatant. The concentration of potassium represents the standard used (20 mM) with magnesium and calcium represented by open squares and circles, respectively. The vertical scale in the left side should be used for Mg and Ca. The concentrations of copper and manganese are represented by closed triangles and circles, respectively. The vertical scale on the right side should be used for Cu and Mn.

the major ions, the concentration of magnesium was constant during the reaction and that of calcium showed little decrease. Because the reaction mixture contained many kinds of anions such as PO_4^{3-} or CO_3^{2-}, the solubility of the calcium ion was low and was saturated in the mixture during the reaction. Among the six transition metal ions, the concentrations of copper, manganese and iron were measured. Copper ion decreased its solubility during the first week of heating. It is noteworthy that this period was the same as that of the appearance of the precipitates. After 1 week of heating, the concentration of copper ion was almost constant. Since iron did not show any detectable values in these samples, the concentration of iron ion was less than 0.01 mM. This would be due to precipitation with anions such as PO_4^{3-} or OH^- in the mixture. The time course of the manganese ion showed almost the same behavior as that of calcium with its concentration reduced by about half after heating 5 weeks.

TABLE I

The time course of the concentrations of each amino acid (a) in the supernatant without hydrolysis and (b) in the hydrolyzed supernatant of the reaction mixture. The value of valine was taken as a standard. The values of decrease were recalculated by the following equation: $100(A_0-A_5)/A_0$. A_0 and A_5 represent the values at 0 h and 5 w, respectively.

(a)

	0 h	8 h	24 h	1 w	2 w	3 w	4 w	5 w	Decrease(%)
Asp	2	2	3	11	19	26	30	31	–
Thr	69	65	65	62	60	59	57	53	23
Ser	134	133	128	118	109	103	95	87	35
Glu	269	252	220	67	23	6	4	3	99
Pro	175	167	164	160	160	161	161	159	9
Gly	47	47	45	46	47	48	48	47	0
Ala	54	54	54	53	53	54	55	53	2
Cys	–	–	–	–	–	–	–	–	–
Val	100	100	100	100	100	100	100	100	–
Met	32	32	31	22	13	5	2	0	100
Ile	24	25	25	26	26	26	25	24	0
Leu	177	175	175	170	172	170	167	163	8
Tyr	48	49	51	45	45	43	40	35	27
Phe	51	55	56	54	53	51	47	44	14
His	24	22	18	12	7	5	2	1	96
Lys	78	75	72	72	69	68	66	61	22
Arg	34	33	32	31	28	27	25	19	44
NH_4	32	89	41	94	130	174	213	271	–

(b)

	0 h	8 h	24 h	1 w	2 w	3 w	4 w	5 w	Decrease(%)
Asp	68	69	69	66	62	59	54	51	25
Thr	62	62	63	62	59	58	55	52	16
Ser	84	83	86	85	81	82	81	73	13
Glu	260	263	261	248	243	245	250	259	0
Pro	171	175	176	180	182	183	186	190	(–11)
Gly	45	44	44	46	47	49	48	48	(–6)
Ala	52	53	52	54	53	53	52	52	0
Cys	–	–	–	–	–	–	–	–	–
Val	100	100	100	100	100	100	100	100	–
Met	33	33	32	30	28	22	18	17	48
Ile	25	25	25	25	26	26	25	25	0
Leu	157	160	162	162	162	161	158	157	0
Tyr	49	51	49	42	43	40	38	31	37
Phe	52	55	53	52	52	49	46	42	19
His	23	22	20	13	8	6	3	2	91
Lys	72	70	72	70	67	68	64	62	14
Arg	31	28	30	29	27	24	21	18	42
NH_4	111	98	101	142	175	205	221	250	–

The time course of the concentrations of each amino acid in the supernatant was studied by using an amino acid analyzer before and after hydrolysis. The value of each amino acid is shown with reference to that of valine (Table I). Decreases due to thermal reactions were seen in the concentrations of Thr, Ser, Glu, Met, Tyr, Phe, His, Lys and Arg, especially in Glu, Met and His (Table I(a)). After hydrolysis, His was not recovered, Met about 50% and Glu was completely recovered (Table Ib). Methionine is easy to decompose by heating. Therefore, by heating for 5 weeks most of the histidine and approximately half of the methionine was thermally decomposed. In addition most of the glutamic acid and about half of methionine was incorporated into soluble polymers. Other amino acids decreased to some extent by heating.

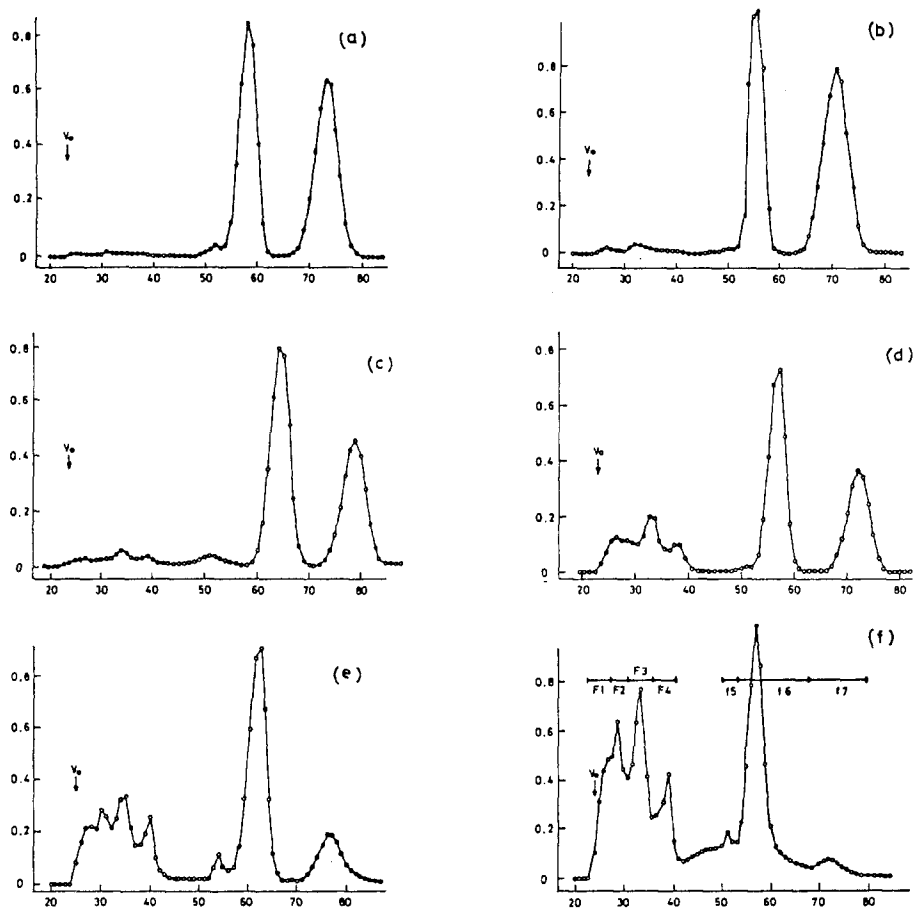

Fig. 3. The elution patterns of the supernatant of the reaction mixture after heating for (a) 0 h, (b) 8 h, (c) 24 h, (d) 1 w, (e) 3 w and (f) 5 w. After being charged on the Bio–Gel P–2 column, they were eluted by 0.01 M NH_4HCO_3 (pH = 8.0 ± 0.5) and the absorbance of the elutants at 275 nm were measured.

An aliquot of supernatant of each sample was then studied with a Bio–Gel P–2 column. Three main peaks and one shoulder were seen in the polymer fraction, namely in the range of elution number between 25 and 45 (Figure 3). These were named F1, F2, F3 and F4 in order of their elution time (Figure 3f). F1 was eluted at the position of void volume, so that its molecular weight was more than 2000. F1 and F2 were eluted closely, but they were clearly distinguished by their absorption spectra. F2 had an absorption maximum near 330 nm but F1 did not. Other fractions also did not have an absorption maximum near 330 nm. Analysis of these fractions following hydrolysis showed that the F2 and the F3 polymers were mainly composed of Glu and 1/10 times amounts of Asp. In addition, they also contained small amounts of Thr, Ser and His: [F2] Glu 901, Asp 87; [F3] Glu 822, Asp 89, Thr 72 (per 1000 res.). Amounts of each of these fractions increased with total amounts increasing up to 50% after heating 5 weeks. The amounts of low molecular weight fractions (in the range of elution number after 50) decreased by heating. The decrease in f7 was most. This fraction proved to be tryptophan by UV-spectrum and comparision of the elution time with an authentic one.

3.2. DEHYDROGENTATION REACTIONS CATALYZED BY THE HIGH MOLECULAR WEIGHT SOLUBLE POLYMER FRACTIONS (HMWSP)

HMWSP was eluted only at the void volume position by the Bio–Gel P–2 column (Figure 4), suggesting that its molecular weight was greater than 2000. Its yield was 0.74 wt% of the amino acids which were originally added to the medium. The total quantity of protein amino acids was about 5 wt% of the HMWSP which consisted of C 29; H 4; N 5; and ash 32 wt%. The absorption spectrum was a gently-sloped one in both the UV and the Vis regions. Some peaks in IR region and excitation and emission spectra were seen (Okihana and Egami, 1979).

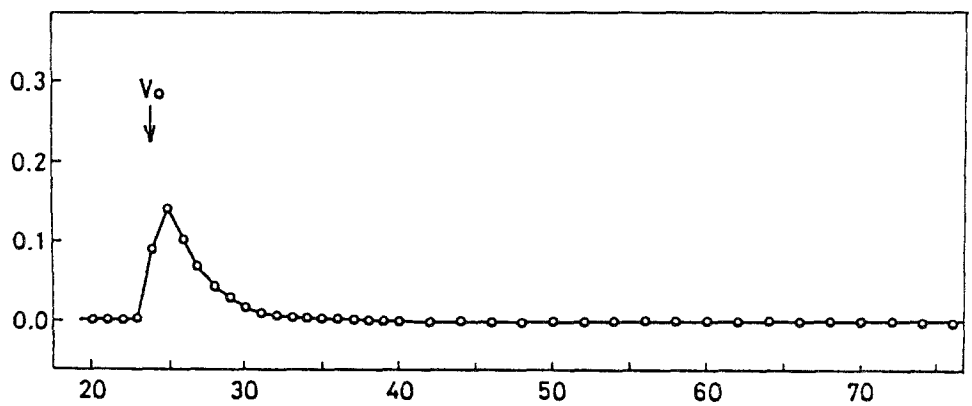

Fig. 4. The elution pattern of the high molecular weight soluble polymers fraction. The elution condition was the same as shown in Figure 3.

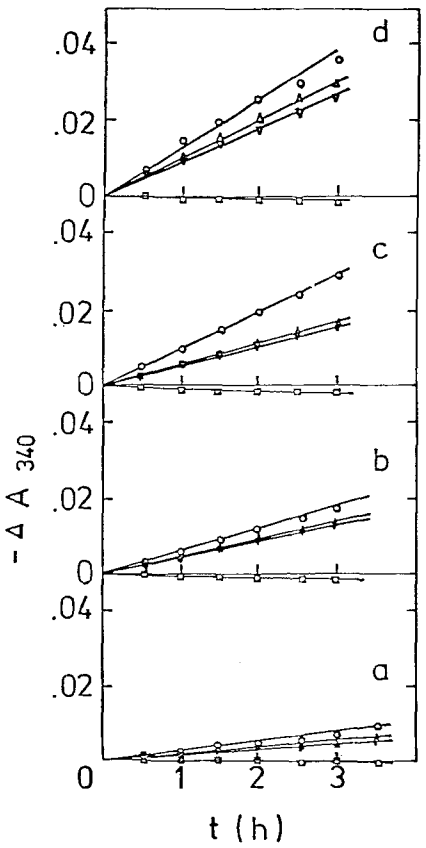

Fig. 5. Dehydrogenation of NADH by changing the concentrations of NADH. The concentrations of NADH were (a) 0.10, (b) 0.20, (c) 0.30, (d) 0.40 mM. Each experiment was composed of 20 μg/ml of HMWSP and NADH (–○–○–); 20 μg/ml of BSA and NADH (–Δ–Δ–); NADH only (–▽–▽–); or buffer only (–□–□–). The system contained 0.05 M of sodium phosphate buffer (pH 7.2).

Dehydrogenation of NADH was studied with variable concentrations of NADH (Figure 5). Bovine serum albumin was used as a control polymer. Each reaction rate was linearly increased by increasing the concentration of NADH. As the observed absorbance range was very small, a spontaneous decrease of NADH was observed. In each case dehydrogenation of NADH was little accelerated with BSA but was accelerated with HMWSP.

The reaction was studied at the fixed concentration of NADH (0.30 mM) with variable HMWSP concentrations. Dehydrogenation was accelerated more by the addition of a higher concentration of HMWSP (Figure 6). The degree of acceleration was in proportion to the amount of HMWSP.

To see the effect of a dissolved oxygen in the reaction solution, reaction mixtures were bubbled with nitrogen gas after three hours. Oxygen did not affect the dehydrogenation rate of NADH (Figure 7). Therefore oxygen is not a hydrogen acceptor in this reaction. This conclusion is also supported by another experiment using the oxygen electrode. The concentration of oxygen in the reaction mixture did not decrease during the reaction.

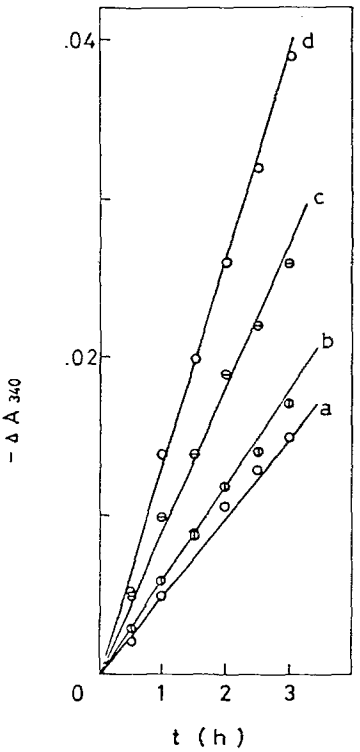

Fig. 6. Dehydrogenation of NADH with variable concentrations of HMWSP. Concentration of NADH was 0.30 mM in the sodium phosphate buffer (0.05 M, pH 7.2). The concentrations of HMWSP were (a) 0, (b) 9, (c) 19 and (d) 46 μg/ml.

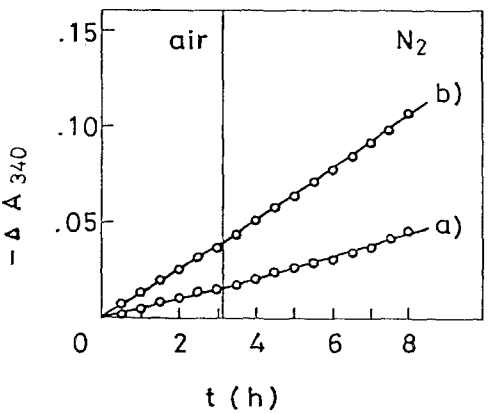

Fig. 7. Dehydrogenation of NADH under the air or the nitrogen gas. After a reaction time of three hours, the reaction mixture was bubbled with nitrogen gas. The concentration of NADH was 0.30 mM in the sodium phosphate buffer (0.05 M, pH 7.2), and those of HMWSP were (a) 0 and (b) 46 μg/ml.

HMWSP did not accelerate the coupled reaction between the dehydrogenation of NADH and a reduction of methylene blue, but did accelerate the coupled reaction between the dehydrogenation of NADH and a reduction of resazurin (Figure 8). This coupled reaction proceeded without HMWSP, and was accelerated by the addition of HMWSP. More precise study will be done to clarify whether HMWSP is one of the constituent parts of the electron transfer chain (NADH → HMWSP → resazurin) or whether HMWSP serves as the catalyst in the coupled reaction between NADH and resazurin.

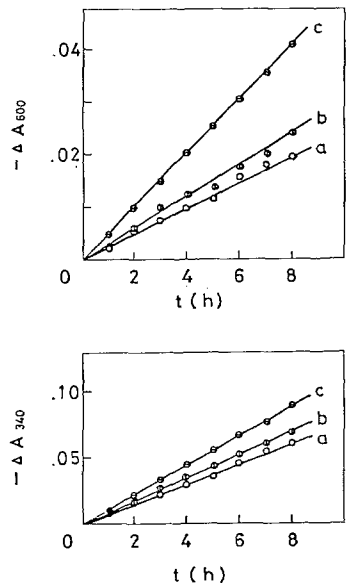

Fig. 8. Coupled reactions between dehydrogenation of NADH and reduction of resazurin with variable HMWSP concentrations: (a) 0, (b) 9 and (c) 19 μg/ml. The concentrations of NADH and resazurin were 0.20 mM and 6 μg/ml in the sodium phosphate buffer (0.05 M, pH 7.2).

It was also shown that this coupled reaction was accelerated a several hundred times more by the irradiation of Xenon lamp.

The dehydrogenation reaction is a very important reaction to acquire energy for living systems. At some stage of chemical evolution a primitive energy transfer system must have been formed. It is said that hundreds of compounds had accumulated in the primitive sea (a primordial soup). NADH and resazurin were used in this study because they were easily available in the laboratory. Any substances in the primordial soup could have been used if these oxidation-reduction potentials are the same value as NADH and resazurin. Prebiotically-formed polymers like HMWSP might have used these compounds to get energy. Of further interest to chemical evolution is the fact that the coupled reaction was enhanced by light.

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