MOLECULAR COMPLEXES OF AMINO ACIDS WITH PORPHYRINS AS POSSIBLE PRECURSORS OF PIGMENT-PROTEIN SYSTEMS

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Abstract. The present communication gives experimental results of the studies of catalytic and photochemical properties of peptide-like compounds containing metalloporphyrins (hemoproteinoids and molecular complexes obtained through adsorption of porphyrins and amino acids on volcanic ash). The data suggest that molecular complexes of amino acids with porphyrins could have evolved in the course of chemical evolution and were intermediated between abiogenically synthesized molecules of amino acids and porphyrins and pigment-protein systems of living organisms.

1. Introduction

In modern living organisms an important role is played by pigment-protein complexes involved in dark and light reactions accompanied by electron transfer. The formation of molecular complexes from amino acids and porphyrins was an essential step in the chemical (prebiological) evolution (Oparin, 1957), that led to the emergence of porphyrin-dependent photosynthesis and respiration. These compounds could have accelerated primary oxido-reductive reactions via catalysis or photochemical activation (Krasnovsky, 1974). Abiogenic synthesis of porphyrin-peptide compounds was proved possible by the experiment on hematin incorporation into thermal proteinoids (Fox and Dose, 1972; Dose and Zaki, 1971) and by our experiments on thermal synthesis in the course volcanic ash adsorption (Kolesnikov and Egorov, 1978a, b). The present communication gives experimental results of our studies of catalytic and photochemical properties of peptide-like compounds containing metalloporphyrins.

2. Methods

Thermal synthesis of porphyrin-peptide compounds on volcanic ashes. In our study ashes from the volcano Tjatja (Markhinin *et al.*, 1975) were used. Samples of the juvenile ash (with particles of 100–200 mesh or less) were quantitatively extracted with benzene-methanol (9:1) and heated at 525°C to remove organic matter (Kolesnikov and Egorov, 1978a, 1979). The treated ash was suspended in an aqueous solution of a mixture of amino acids (5 g of ash, 100 ml of solution of eight amino acids in a concentration of 5×10^{-3} *m* each, pH 7.0–7.5). L-amino acids produced by Reanal Co (lysine, arginine, proline, isoleucine, glycine, β-analine, aspartic and glutamic acids)

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were used. The suspension was mixed on a magnetic mixer and left for 18 h for the adsorption of amino acids. The ash was then removed by centrifuging and dried at 45°C. The dried ash was then suspended in 200 ml of 5×10^{-4} *m* solution of protoporphyrine-9 in benzene containing 10% of methanol. After adsorption of the pigment, the ash was separated from the solvent and again dried. The samples containing 8.2% of water were heated in sealed evacuated ampules for 240 h at 96° or 120 h at 150° (Kolesnikov and Egorov, 1978a, b). Then they were extracted with azeotropic mixtures of benzene-methanol (3:2) and acetonitrile-benzene-water (2.3:6.9:0.8) in the Soxhlet apparatus and 0.1 NNaOH. Alkaline extracts were dialyzed and purified by gel filtration on Sephadex G–25 and G–50.

Preparation of proteinoids containing metalloporphyrins. Dry mixtures of L-amino acids containing 3% protoporphyrin-9 or its complexes with Cu(II), Zn(II) and Fe(III) were heated in vacuo or in the nitrogen atmosphere for 4 h at 150–170°C. Two initial mixtures of amino acids were used in our experiments:

1. 4:2:1:3 (glutamic acid, aspartic acid, lysine and mixture of 7 amino acids in equimolar proportions: histidine, arginine, alanine, glycine, proline, isoleucine, and phenylalanine);
2. 4:4:1:3 (glutamic acid, aspartic acid, lysine and mixture of 9 amino acids in equimolar proportions: histidine, cystein, glycine, alanine, proline, valine, leucine, isoleucine and phenylalanine).

In some experiments the reaction mixture was supplemented with polyphosphate, polyphosphoric acid and volcanic ash (to increase the yield of proteinoids). Polyphosphate was obtained by method of Osterberg and Orgel (1972). Reaction products were purified on Sephadexes G–25 and G–50, and in subsequent experiments only the material with a molecular weight of over 5000 was used. Eluates were dialyzed and evaporated in vacuo.

Molecular weights of proteinoids were measured by gel filtration on Sephadex G–50 in 0.1 N KOH or 0.05 *M* Tris-HCl-buffer containing 0.2% sodium dodecyl sulfate (pH 8.0). Amino acid composition was determined by Watmann 3 mm paper chromatography that followed hydrolysis (6 N HCl for 48 h) and hydrolyzate purification on the cation exchange resin KY–2. Extinction coefficients were measured as follows: (D_{400})-optical density of 0.1% proteinoid solutions at 400 nm in a 1 cm thick cuvette and biuret colouring (D_{300})-optical density at 300 nm of 0.05% proteinoid solutions after an addition of biuret reagent (in a 1 cm thick cuvette) taking into consideration absorption of the proteinoid and biuret reagent.

The iron content in hemoproteinoids was measured colorimetrically using 1,10-phenantroline and on that basis, the content of ferroporphyrins was calculated. Hemoproteinoids were boiled with 12 N HCl and 10% hydroxyl-amine was added to the acid solution obtained for reduction of Fe(III) to Fe(II).

Peroxidatic activity of hemoproteinoids was assayed according to the method with pyrocatechol (Petrochenko and Kolesnikov, 1966). The presence of catalatic activity was judged according to the decrease in the amount of H_2O_2 by the iodine-starch

color reaction (Kolesnikov, 1958). Oxidatic activity of proteinoids was determined with respect to NAD $\cdot H_2$ oxidation (Petrochenko and Kolesnikov, 1965).

To study photochemical activity, the solutions were illuminated with incandescence lamps (300–500 W) through a two-lenzed condenser and water filter. The experiments were carried out in Tunberg tubes at 19–21°C in the presence of a detergent to obtain translucent proteinoid solutions at pH 3.5–7.2. The experimental solutions were made of proteinoid (0.1 %, 1 ml), Triton X–100 (5 %, 1 ml), electron acceptor (0.25 ml), ascorbic acid (10^{-2} *m*, 1 ml) and buffer solution (to bring the final volume to 5.0 ml). Riboflavin (5×10^{-4} *M*, 1 ml) and versene (1 %, 0.5 ml) were added to activate photoreduction reactions of hemoproteinoids. Dark controles and light controles without proteinoids were made to determined changes in the optical density of experimental solutions as compared with controls.

3. Results and Discussion

The porphyrin-peptide complex obtained through adsorption on ashes had a molecular weight of 10 000, contained Cu(II)-protophorphyrin-9 (according to ESR–data) and showed an absorption maximum near the Soret band (410 nm). The molecular weights of hemoproteinoids ranged from 10 000 to 18 000, and the ferroporphyrin content amounted to 10.0–16.0 %. the resultant compounds contained all the amino acids of initial mixtures. Preliminary experiments showed that the properties of proteinoids obtained in vacuo and in the N_2 atmosphere were identical; however, the yield of proteinoids in vacuo was higher. The pattern of the synthesis and properties of hemoproteinoids containing Fe(III)-protoporphyrin-9 are given in Table I.

Metalloporphyrins were probably linked covalently: they were extracted with a strong acid only (12 N HCl, 6 h at 80°C). The resultant porphyrin-peptide complexes oxidized pyrocatechol in the presence of H_2O_2 (peroxidase properties) and showed photochemical activity.

Peroxidatic and oxidatic activities of hemoproteinoids were superior to the corresponding activities of hematin (Table II). Catalatic property of hemoproteinoids was less than that of hematin, this being in agreement with the literature data (Dose and Zaki, 1971).

Peroxidase activity of proteinoids obtained under different conditions is shown in Figure 1. The solutions are balanced with respect to iron concentration. Previously, catalytic activity of proteinoids was reported (Rohlfing and Fox, 1969; Dose, 1974).

In our experiments hemoproteinoids and proteinoids containing Zn(II)-protoporphyrin-9 sensitized pyrocatechol photooxidation and photoreduction of methyl red with ascorbic acid (Figures 2, 3). Photoreduction of methyl red was activated with reduced 2.6-dichlorphenol-indophenol (2.6–DPIP), as was described for porphyrins (Vernon, 1959).

The resultant proteinoids catalyzed also photoreduction of 2.6–DPIP and photooxidation of pre-reduced 2.6–DPIP as well as photoreduction of tetrazolium compounds with ascorbic acid (Kolesnikov *et al.*, 1979a, b). The proteinoids catalysed

TABLE I
The pattern of the synthesis and properties of hemoproteinoids obtained in the different conditions

The pattern of the synthesis	Yield, (w %)	D_{400}	D_{300}	Molecular weight
4:2:1:3				
150°	10.2	0.57	0.44	10 000–16 000
150°. in presence of 4 parts of volcanic ash	14.7	0.64	0.43	
150°, in presence of polyphosphoric acid	16.3	0.67	0.47	
150°, in presence of 4 parts of polyphosphate	13.2	0.66	0.39	
170°	18.2	0.79	0.38	10 000–17 000
170°, obtained in the absence of glycine	10.4	0.39	0.43	
170°, enriched by glycine	16.3	1.22	0.42	
170°, enriched by histidine	17.4	0.90	0.49	
4:4:1:3				
170°	8.0	0.61	0.32	10 000–18 000
170°, in presence of 4 parts of polyphosphate	14.6	0.49	0.24	
170°, in presence of 4 parts of volcanic ash	10.5	0.18	0.34	
170°, in presence of pholyphosphoric acid	13.2	0.45	0.29	

TABLE II
Comparison of the catalytical properties of Hematin and hemoproteinoids (0.05 M K-phosphate buffer, pH 7.2; the solutions are balanced with respect to the iron concentration)

	Change in the optical density of experimental solutions as compared with the control		
	Catalatic activity in the presence of imidazole (for 25 min)	Oxidation of NAD $\cdot H_2$ in the presence of resorcine (for 60 min)	Peroxidatic activity by oxidation of pyrocatechol (for 25 min)
Hematin 8×10^{-5} M)	0.65	0.15	0.11
Hemoproteinoid 4:4:1:3 (5×10^{-2} %)	0.30	0.42	0.51
Hemoproteinoid 4:2:1:3 (5×10^{-2} %)	0.22	0.50	0.53
Proteinoid 4:2:1:3 (5×10^{-2} %)	0.00	0.00	0.00

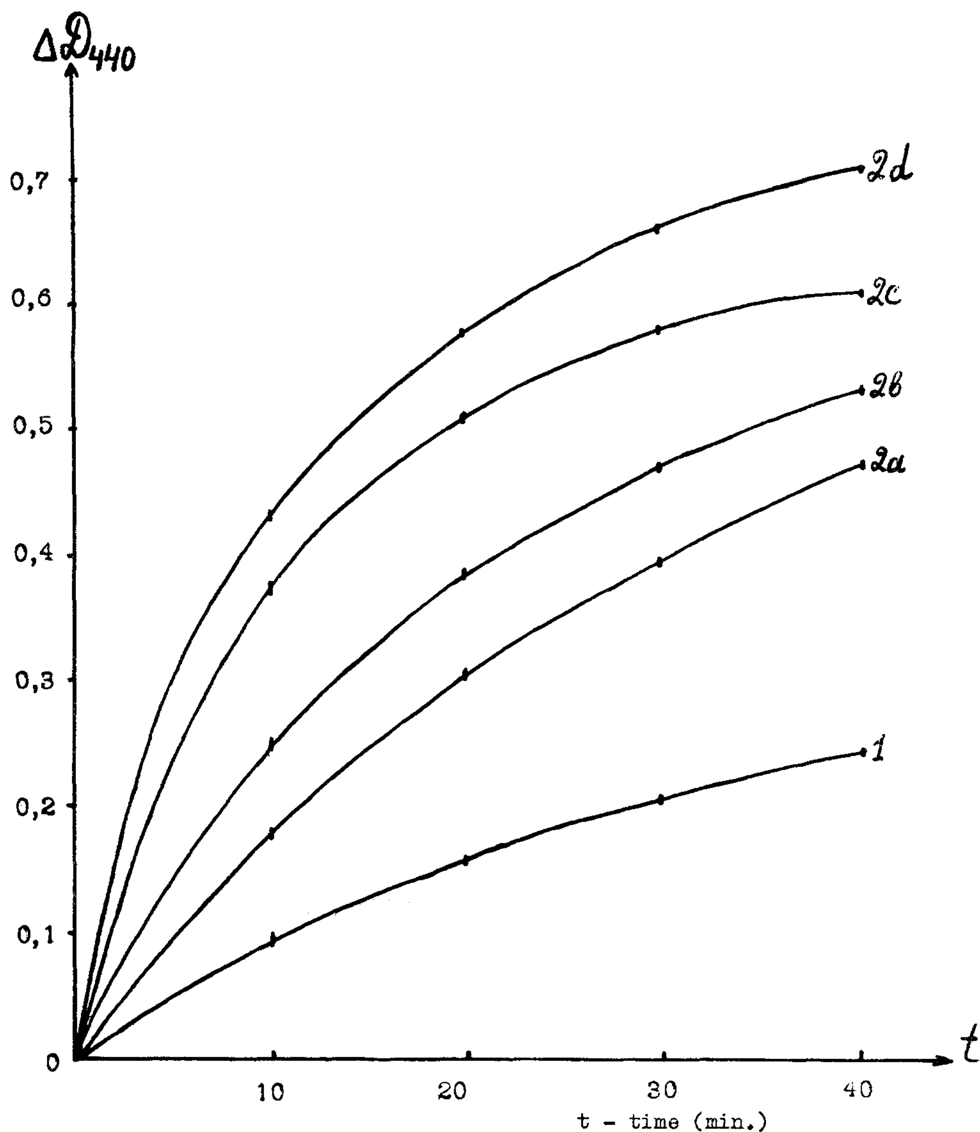

Fig. 1. Peroxidase activity of hematine (1) and hemoproteinoids 4:2:1:3 obtained under different conditions (2). The solutions are balanced with respect to iron concentration. a: 150°, b: 170°, c: 150° (obtained in the presence of polyphosphate); d: 150° (obtained in the presence of volcanic ash).

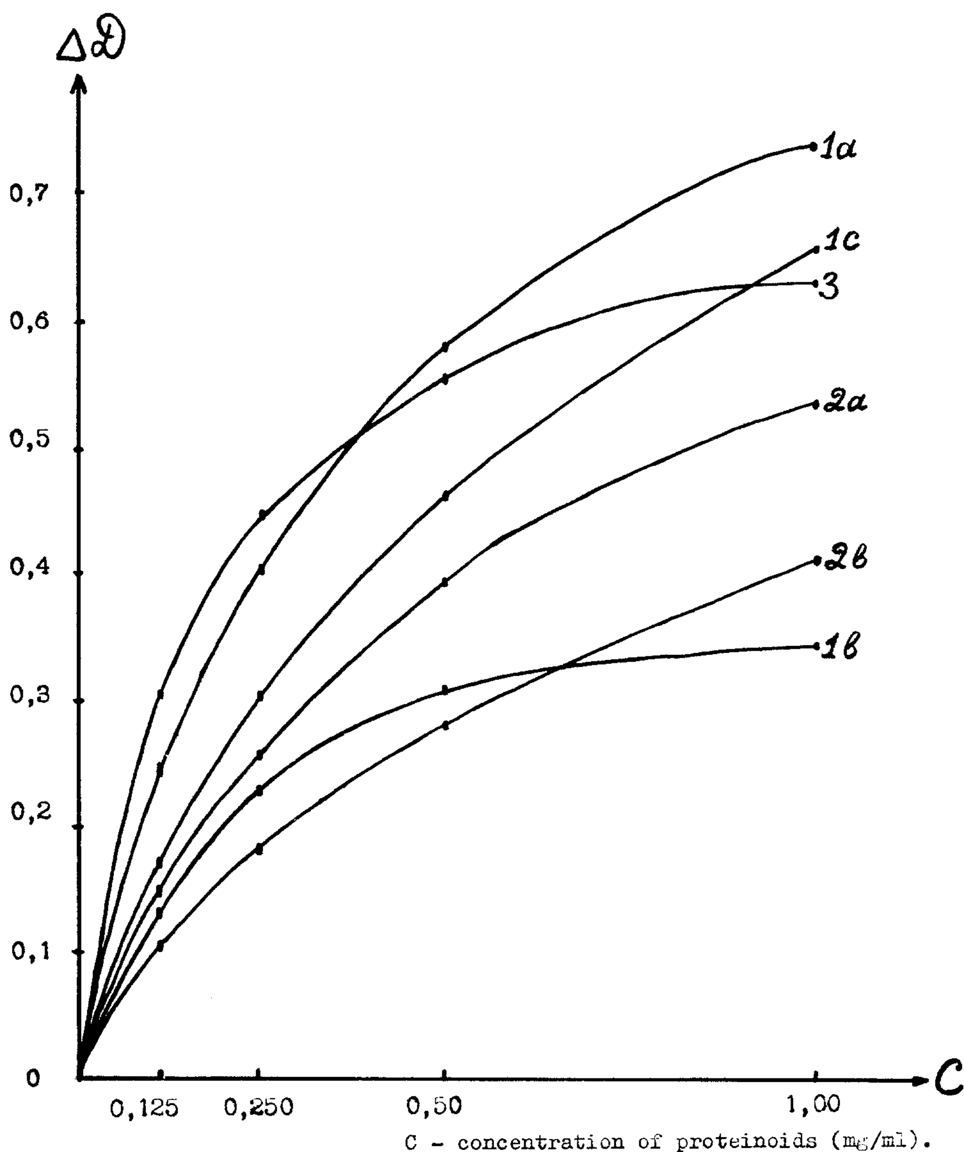

Fig. 2. Photochemical activity of proteinoids 4:2:1:3 (obtained at 150°) containing Fe(III)-protoporphyrin-9 (1) and Zn(II)-protoporphyrin-9 (2,3). 1,2: Photoreduction of methyl red with ascorbic acid in vacuo; 3: Photooxidation of pyrocatechol (pH 7.2 in presence of 0_2); a: pH 5.2; b. pH 7.2; c: pH 7.2 in presence of reduced 2.6–DPIP). △D: Change in the optical density of experimental solutions as compared with the control.

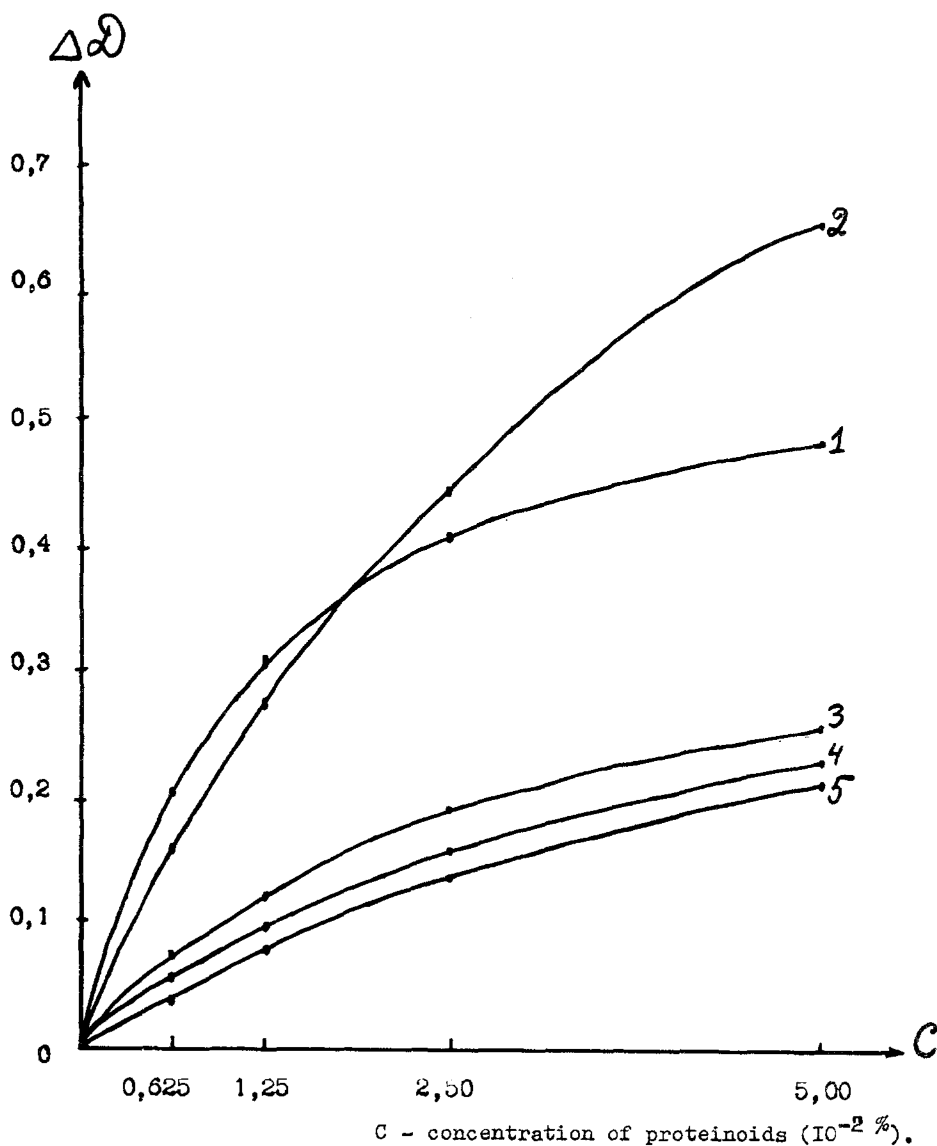

Fig. 3. Photoreduction of methyl red with ascorbic acid (in vacuo) sensitized by hemoproteinoids 4:2:1:3 (obtained at 170°) in the presence of reduced 2.6–DPIP (5×10^{-2} M, 0.5 ml). 1: hemoproteinoid 4:2:1:3; 2: Glycine enriched hemoproteinoid; 3: hemoproteinoid obtained in the absence of glycine; 4: proteinoid 4:2:1:3 (obtained in the absence of Fe-protoporphyrin-9); 5: glycine heated alone (4 h at 170°) in the presence of polyphosphoric acid.

also photooxidation of ascorbic and oxalic acids (in the presence of O_2).

As known, the photosensitizing effect of various compounds is based on their capacity for photoreduction or photooxidation. We carried out an investigation of photoreduction of hemoproteinoids. Preliminary experiments demonstrated that dark reduction of hemoproteinoid by sodium dithionite led to an increase in the intensity of the absorption band at 575 nm and appearance of maximum at 550 nm in the absorption spectrum; therefore, that was the case of iron translation into a bivalent state. There are literature data indicating photoreduction of Fe(III)-complexes of pheophytins and pheophorbids accompanied by a change in the valence of iron atoms (Aschkinasi *et al.*, 1955). Direct photoreduction of hemoproteinoid (in 0.05 *M* Tris-HCl-buffer, pH 8.6 in vacuo) was not advanced, and riboflavin (5×10^{-4} *M*, 1 ml) accelerated the reactions. Photoreduction was monitored with respect to an increase in the optic density at 550 nm. As is the case with cytochrome *c* (Krasnovsky and Mikhailova, 1970) our experiments showed a transfer of an electron from the donor components of the proteinoid molecule to the iron atom (riboflavin acting as an intermediate electron carrier). Histidine-enriched hemoproteinoid (25% histidine in the initial mixture of amino acids) was reduced in a more active way, while hemoproteined obtained in the absence of glycine displayed low activity even in the presence riboflavin. Photochemical properties of proteinoids are believed to be associated with the presence of glycine which under the conditions of thermal polycondensation forms a colored pigment (Weber *et al.*, 1968).

It is very likely that the glycine pigment is an essential electron-donor component of the hemoproteinoid molecule affecting significantly iron reduction in the prosthetic group. Hemoproteinoid obtained in the absence of glycine was inactive in the reaction of methyl red photoreduction as was the case with the proteinoid containing no metalloporphyrins. glycine enriched hemoproteinoid (25% glycine in the initial mixture of amino acids) showed the highest activity in this reaction (Figure 3). Ferroporphyrins were photochemically inactive (Vernon, 1961); However, their incorporation into the structure of proteinoids allowed photoreduction of the iron atom and photochemical oxidoreductive reactions. Such reactions with an involvement of primary photosensitizers were in all probability evolutionary precursors of porphyrin-dependent photosynthesis. These findings are of certain interest in terms of our knowledge of processes of chemical evolution on the Earth. The data suggest that molecular complexes made of amino acids and porphyrins could have evolved in the course of chemical evolution and were intermediates between abiogenically synthesized molecules of amino acids and porphyrins and pigment-protein complexes of living systems.

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