PROTEINOIDS AS COMPLEXES OF POLYAMINO ACIDS WITH MELANOIDINS

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Abstract. Proteinoids have been demonstrated as complexes of amino acid polymers with melanoidin pigments. Some physico-chemical properties of proteinoid pigments were studied in comparison with the standard melanoidins. Proteinoid pigments were able to enhance oxidoreduction and hydrolysis reactions, and their activity was comparable with the activity of the corresponding polyamino acid components or even of the entire proteinoids. The pigmented proteinoids had relatively strong ESR signal indicating the presence of free radicals into melanoidin components. Hypothetical participation of proteinoid melanoidin pigments in prebiotic evolution is discussed.

1. Introduction

Proteinoids – thermal copolymers of amino acids – have been suggested as a plausible model for the study of prebiotic evolution. Since early 60s when the first experiments were carried out, proteinoids have been shown to enhance the rate of several reactions analogous to the enzymic biochemical processes (for review see Fox and Dose, 1977). This ability of proteinoids was usually associated with their protein-like moiety; in the case of redox-reactions of hemoproteinoids it was also associated with the prosthetic group of heme (Dose and Zaki, 1971; Masinovsky, 1980). However, practically all known proteinoids contain pigments (being yellow up to dark-brown). Very poor information concerning the nature and properties of proteinoid pigments is available. Heinz with collaborators (Heinz *et al.*, 1979) found traces of pteridines and flavines among them.

The aim of the present work was to separate proteinoid pigments from proteinoid amino acid polymers and to examine some of their physico-chemical and catalytic properties.

2. Materials and Methods

Proteinoids were synthesized from dry homogenous mixtures of amino acids by heating at 185 °C for 6 hr in the presence of nitrogen. Polymeric compounds were isolated from the total product of thermal synthesis by dialysis against water for

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72 hr and then lyophilized. Pigments were isolated from proteinoids either by hydrolysis of amino acid polymers with 6 M HCl at 110 °C for 24 hr or by extraction with dimethyl sulfoxide.

The content of peptide-like amino acid polymers was determined by four methods: biuret method (Itzhaki and Gill, 1964), method with Coomassie blue G-250 (Scopes, 1982), Lowry's (Lowry *et al.*, 1951) and Greenberg's method (Greenberg and Gaddock, 1982).

Colorimetric measurements have been corrected by taking into consideration the absorbance of the proteinoid pigment itself as well as of the coloured substances yielded from the interaction of melanoidins with the reagents used for the peptide detection.

The heterogeneity of proteinoids and their hydrolyzates was determined by gel-chromatography on Sephadex G-50 and TSK-gels HW-40 and HW-50 with 0.05 M NaOH as eluent.

Standard melanoidins were synthesized by heating of equimolar mixture of glycine and glucose at 80 °C for 21 hr. Spectroscopic measurements were carried out by using Beckman spectrophotometer model 35 (UV- and VIS-spectra), Specord M-80 (IR-spectra), and Varian E-9 (ESR-spectra).

The catalytic activity of entire proteinoids, proteinoid amino acid polymers and proteinoid pigments was examined in the following reactions.

Oxidoreduction

(a) The mixture of 2.5 mL of 0.02 M oxalic acid, 0.1 mL of 0.01 M $KMnO_4$ and 0.1 mL of examined substance (entire proteinoid or the corresponding amino acid polymers or the corresponding pigments) (20 mg mL^{-1}) was placed into cuvette. The reaction rate was measured photometrically at 525 nm.

(b) The mixture of 0.16 mL of 0.03 M sodium pyruvate, 0.1 mL of 0.006 M NAD(H) (pH 8.0), 0.1 mL of lactate dehydrogenase (Reanal, 2×10^{-3} mg mL^{-1}) or the examined substance (20 mg mL^{-1}) and 2.6 mL of 0.1 M phosphate buffer (pH 7.0) was placed into the cuvette. The reaction rate was measured photometrically at 340 nm. Controls without any catalytic substance were carried out to exclude artefacts due to an oxidation of NADH by 0_2.

Hydrolysis

(a) The mixture of 0.01 mL of 0.2 M glucose 6-phosphate, 0.1 mL of acidic phosphatase (Reanal, 1 mg mL^{-1}) or the examined substance (20 mg mL^{-1}) and 1.0 mL of 3 M acetate buffer (pH 5.0) was incubated for 15 min at 37 °C. Released phosphate was detected photometrically at 735 nm (Rathbun and Betlach, 1969).

(b) The mixture of 2 mL of phenolphthalein monophosphate (2 mg mL^{-1}) and 0.1 mL of acidic phosphatase (Reanal, 1 mg mL^{-1}) or the examined substance (20 mg mL^{-1}) was incubated at 37 °C for 15 min. Then 1 mL of 0.5 N NaOH was added (up to pH 9.0) and the released phenolphthalein was detected photometrically at 550 nm.

3. Results and Discussion

Three groups of proteinoids have been synthesized: neutral (from the equimolar mixture of 18 amino acids; Masinovsky, 1984), basic (from the same mixture with extra 50% of lysine) and the aromatic-free (from the same mixture except tyrosine, tryptophan and phenylalanine). All of them were of brown colour.

The content of amino acid polymers measured in the total product of the thermal synthesis is presented in Table 1.

TABLE I

The peptide content (%) of the total products of thermal polymerization of amino acids (calculated from the human serum albumin calibrating plot)

Type of proteinoid	Method of determination			
	Biuret	Lowry's	Coomassie G-250	Greenberg's
neutral	5.2	3.4	2.0	2.0
basic	2.2	4.7	2.2	2.4
aromatic-free	*	2.0	2.9	*

* Determination was impossible due to the strong absorption of proteinoid pigments.

The yield of the substances of the peptide nature in the total product is relatively low – no more than 5%. The differences between the data obtained by various methods could result from the fact that in contrast to the Lowry's and biuret methods, the Greenberg's method does not detect free amino acids and, therefore, is the most suitable for the detection of low amounts of the protein-like substances at the presence of free amino acids and other relative compounds.

Fox and Harada (1966) have reported substantially higher yields (10–40%). They have also detected the peptide bonds in proteinoids but the yields were calculated by them from the dry weights of products (not from the peptide-like content, as we did) which resulted in the substantially higher values.

The portion of the substances of peptide nature in relatively pure polymeric fraction obtained by dialysis of the total product ('pure' proteinoids, i.e. free of the low molecular products of thermal polycondensation, of free amino acids, etc.) was found to be considerably higher (up to 70%) when measured by Greenberg's method.

By gel-chromatography (Figure 1) proteinoids studied in this work were identified to be relatively heterogenous mixtures of polymers of molecular weight from 3000 to 10 000 (Table II).

TABLE II

Composition of proteinoids of various types

Type of proteinoid	Molecular weight of the components				
Neutral	3600	6500	8300	9700	
Basic	3400	4700	5500	6500	9700
Aromatic-free	3800	6800	9800		

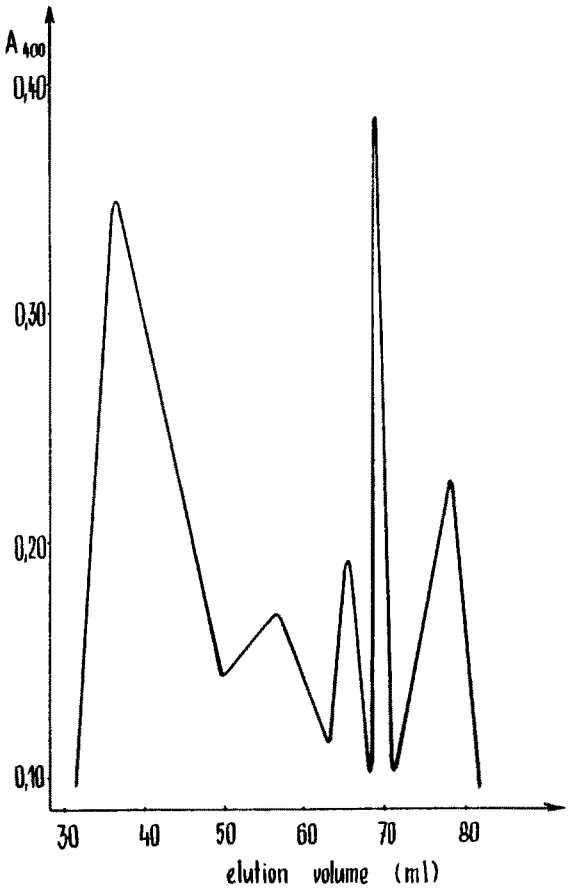

Fig. 1. Gelchromatography of the basic proteinoid. TSK-gel HW-40; eluent: 0.05M NaOH.

After hydrolysis of the amino acid polymers three fractions of proteinoid pigment were obtained by chromatography on TSK-column (800, 1200, 2800).

Isolated proteinoid pigments were identified by comparison with standard melanoidins. It was shown that both groups of pigments are partly soluble in water, practically insoluble in acids and fully soluble in 0.5 M NaOH. Non-polar organic solvents were practically inefficient, while polar organic solvents such as ethylene chlorohydrin and especially dimethyl sulfoxide dissolved them by 100%. These results brought the first evidence of the melanoidin nature of proteinoid pigments.

Further evidence was obtained by UV-, VIS- and IR-spectroscopy (Figure 2). Proteinoid pigments as well as standard melanoidins exhibit efficient absorption in the visible region which increases strongly in UV, both being without characteristic bands. The similarity of IR-spectra of the two groups of pigments (including the position of characteristic absorption maxima – Figure 2) also supports melanoidin nature of proteinoid pigments.

Figure 3 demonstrates ESR spectrum of the pigmented proteinoid. ESR cha-

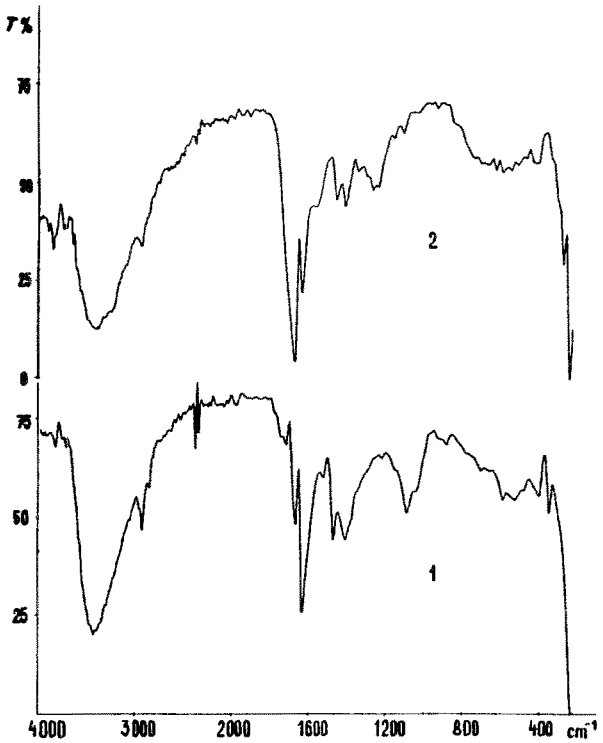

Fig. 2. IR-spectra of the neutral proteinoid pigment (2) and standard melanoidins (1). Potassium bromide pellets. SPECORD M 80.

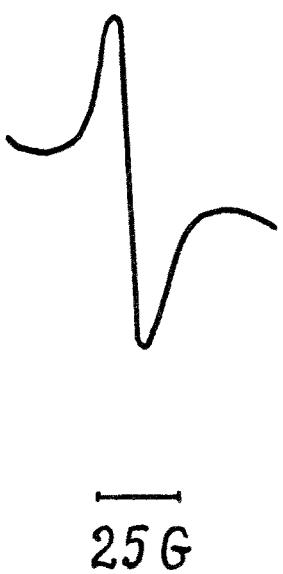

Fig. 3. ESR-spectrum of pigmented proteinoid. Varian E-9.

TABLE III

The kinetics of electron transfer from oxalic acid to potassium permanganate in the presence of proteinoids or their components (detected photometrically as absorbance at 525 nm[a])

Time (min)	Examined substance						
	Without catalyst	Neutral proteinoid			Basic proteinoid		
		Entire	Pigment	Amino acid polymer	Entire	Pigment	Amino acid polymer
0	1.310						
0.5	1.300	1.229	1.202	1.270	1.215	1.168	1.253
1.0	1.283	1.105	1.076	1.203	1.077	1.008	1.150
2.0	1.236	0.926	0.850	1.076	0.875	0.781	1.020
3.0	1.205	0.788	0.695	0.960	0.750	0.672	0.859
4.0	1.182	0.675	0.583	0.830	0.598	0.586	0.762
5.0	1.158	0.607	0.515	0.735	0.524	0.480	0.684
6.0	1.136	0.548	0.457	0.686	0.465	0.443	0.619
20.0	0.715	0.250	0.163	0.289	0.246	0.097	0.308
45.0	0.014	0.016	0.013	0.015	0.018	0.010	0.016

[a] With the error $\leqslant 6\%$.

racteristics were calculated as following: ΔH = 8G, g = 2.003. Non-pigmented proteinoids did not give such a signal under the same conditions (room temperature). UV irradiation at low temperature (up to -196 °C) induced an increase of ESR signal which readily relaxed to the starting level with the increased temperature.

Catalytic properties of proteinoids and their two components (amino acid polymers and melanoidins) have been examined in further experiments. Two model systems were used for the study of redox transformations; oxalic acid - potassium permanganate and sodium pyruvate - NAD(H) (lactate dehydrogenase system).

The results obtained for the first system are presented in Table III.

As can be seen from data obtained for both neutral and basic proteinoids, the rate of electron transfer was higher in the presence of proteinoid melanoidins than that with the corresponding amino acid polymers and even than that with the entire proteinoid. The data presented in Table III were corrected by taking into account an additional direct reduction of permanganate by the pigments which have been shown to take place in the absence of donor in the mixtures of melanoidins with thiazolyl blue 3(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazoliumbromide) or NAD (Telegina *et al.*, 1988).

The results obtained for the system pyruvate - NAD(H) are presented in Table IV. The results obtained with the lactate dehydrogenase system (Table IV) indicate that although the activity of the neutral proteinoid as well as of its components was about 4–5 orders of magnitude lower when compared with lactate dehydrogenase, within 3 hours the reaction reached the same level of NAD as the process catalyzed by the enzyme. In the absence of catalyst any oxidation of NADH was not detected within the same time interval.

TABLE IV

Activity of lactate dehydrogenase, neutral proteinoid and its components in electron transfer from NADH to pyruvate (detected photometrically at 340 nm)

Examined substance	$\mu mol\ mg^{-1}\ min^{-1}$
Lactate dehydrogenase	16900
Neutral proteinoid	
- Entire	0.643 ± 0.032
- Pigment	0.804 ± 0.040
- Amino acid polymer	0.643 ± 0.032

Hydrolytic activity of proteinoids and their components on glucose-6-phosphate and phenolphtalein monophosphate as substrates was studied in comparison with acidic phosphatase. The results obtained for the system with glucose-6-phosphate are presented in Table V. Both basic and neutral proteinoids, as well as their components alone were catalytically active in splitting glucose-6-phosphate (Table V), melanoidins of the neutral proteinoid to be the most active. The activity of these substances was about 100 times lower when compared with the acidic phosphatase. Similar results have been obtained with phenolphthalein monophosphate.

These values are comparable with our previous results (Masinovsky *et al.*, 1980), as well as with the data of Oshima (1968) who had detected the proteinoid hydrolytic activity on p-Nitrophenylphosphate to be about 1000 times lower when compared to a highly purified alkaline phosphatase.

Thus the data mentioned above, as well as the results obtained from gel-electrophoresis (Masinovsky *et al.*, unpublished) indicate proteinoids as stable complexes of amino acid polymers with melanoidin pigments.

Melanoidins are dark-coloured heterogenous pigments containing aromatic and N-containing heterocycles, carbonyl and carboxyl groups as well as the system of

TABLE V

Splitting of glucose-6-phosphate by acidic phosphatase, proteinoids and their components (detected photometrically at 735 nm)

Examined substance	$\mu mol\ mg^{-1}\ min^{-1}$
Acidic phosphatase	131.0
Basic proteinoid	0.992 ± 0.059
- Entire	
- Pigment	0.589 ± 0.042
- Amino acid polymer	0.837 ± 0.050
Neutral proteinoid	
- Entire	0.899 ± 0.049
- Pigment	1.240 ± 0.078
- Amino acid polymer	1.116 ± 0.081

conjugated double-bonds. Melanoidins are usually synthesized by the reaction of amino acids with reducing sugars (Ellis, 1959). However, they can be also obtained through the interaction of practically any of the compounds containing carbonyl and amino groups in a broad interval of temperature and humidity. Melanoidins are believed to have an important role as organic polyfunctional matrices in prebiotic evolution (Nissenbaum *et al.*, 1975; Telegina *et al.*, 1980, 1988).

Besides the activities studies in this work, melanoidins synthesized abiogenically by UV-irradiation of the aqueous mixtures of acetaldehyde (2.5%) and ammonium salts (1.5%) have been demonstrated to be able to catalyze the photochemical synthesis of short peptides from diluted aqueous solutions of alanine (Telegina *et al.*, 1982).

It is commonly assumed that prebiotic systems were strongly illuminated by solar radiation with a great deal of UV-rays which were efficiently absorbed by melanoidins. Under those conditions their photosensitizing activity might play a substantial role in prebiotic catalysis. An intensive fluorescence of melanoidins at 400–500 nm has been detected (Telegina *et al.*, 1980; Lozovaya *et al.*, 1989), indicating their photochemical ability. In agreement with our results the activity of the pigmented proteinoid in the reaction of decarboxylation has been shown to increase significantly under illumination (Wood and Hardebeck, 1972). And finally, the participation of the proteinoid melanoidin pigments in the photochemical processes catalyzed by hemoproteinoids and by porphyrin-proteinoid complexes (Masinovsky *et al.*, 1989) can not be also excluded.

Our results indicating ESR-signal in the pigmented proteinoid (but not in the non-pigmented one) are consistent with the data (Nissenbaum *et al.*, 1975) concerning the presence of free radicals in melanoidins. Thus, melanoidins might be a trap for free radicals including the photoinduced ones and, therefore, protect proteinoids against UV-photodestruction which might have been an important factor in prebiotic environment.

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