SEPARATION OF HEMOGLOBIN INTO HEMIN AND GLOBIN BY MEANS OF CARBOXYMETHYLCELLULOSE

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SUMMARY

The kinetic patterns of hemin and globin sorption on carboxymethylcellulose (CMC) have been studied. The main kinetic and sorption parameters of the process of globin sorption on CMC have been determined. Dependencies of the sorption values of hemin and globin on their concentration in the solution and on temperature have been shown.

Introduction

It is known that hemoglobin, the main protein of the blood-forming elements of the slaughter blood can be successfully used in the food and medical industries (Kern, 1981). However, specific color and taste of hemoglobin hamper its wide application as a source of food protein. In this connection, nowadays several methods of hemoglobin clarification have been developed, among which the most promising one being the sorption method of hemin extraction after dissociation of hemoglobin in acid water-based solutions (Marc et al., 1978). Activated carbon, zeolite, carboxymethylcellulose may be used as sorbents.

In spite of the already existing detailed description of the process of CMC-based hemin extraction from acid solution (Sato et al., 1981), scientific literature practically does not define sorption characteristics of this sorbent. In connection thereof , the purpose of this paper was to study sorption of hemin and globin on CMC, the process being in wide use in the food industry.

Materials and Methods

For experimental purposes the following researh objects were chosen: carboxymethylcellulose, used in the food industry, hemin-base and hemoglobin (the SERVA Company).

A 5% water solution of hemoglobin was acidified to pH 3.0 with 1 N HCl, which was followed by addition of an equal amount of a 1% water solution of CMC. The obtained substance was mixed at 10°, 20°, 30°C during 30-60 min., and after that the formed sediment containing CMC with hemin was separated by means of centrifugation. The initial and final concentration of protein in solution was determined by Lowry (1951), hemin content - by direct spectrophotometry of solution at 405 nm (maximum of absorption constituting 0.1% of hemoglobin solution). The sorption value of components was determined by the difference of absorption values of initial and final solution on spectrophotometer SF-46. Constants in the Lengmure's equation were computed by the least squares method after linearization of this equation in the system of reverse coordinates.

Results and Discussion

During the study of sorption of separate components it turned out that isolated hemin at pH 3.0-3.5 practically does not sorb on CMC in water solutions of different concentrations, and that its sorption on this sorbent starts only when some other protein is added to the globin solution, e.g. sorption of hemin on CMC apparently takes place only after formation of a certain protein layer on CMC, which stimulates sorption of hemin, e.g. formation of a type of "sandwich" is observed: CMC-protein-hemin.

It was established that for achievement of full saturation of CMC for hemin and protein at least 35-40 minutes are required, time of CMC saturation being in the least degree dependant on temperature in case of hemin (Fig. 1), however, showing vivid dependence in case of protein (Fig. 2).

The rate of protein sorption at different temperatures, determined by the well known method (Beresin et al., 1976) and energy of the process activation, determined by the Arrhenius equation, are given in table 1.

Fig. 1 and 2 show sorption curves for hemin and globin as dependent on their concentration in solution. It is interesting to note that sorption of hemin on CMC is practically in linear dependence on its concentration in solution, while sorption of globin acquires hyperbolic dependence and resembles classic Lengmure's curves:

$$E = ABC/1 + BC \quad (1)$$

where E - is sorption ability of CMC in g/g, determined under experimental conditions according to the formula:

$$E = (C_o - C)/M \quad (2)$$

where C_o - is initial concentration of protein in g/l; C - is concentration of protein at the present moment in g/l; M - amount of CMC, g; A - constant value, characterizing concentration of protein when it fills all the active centers of the sorbent, g/g; B - constant of the adsorption process equilibrium, l/g.

By knowing protein concentrations in a solution and experimentally determining E by formula (2), we can calculate constant A and B in the equation (1), having transformed them in linear forms of the type:

$$E/C = 1/AB + 1/AC \quad (3)$$

Values of constants A and B are presented in table 1.

By comparing the sorption curves of hemin and globin we can conclude that at increase hemoglobin concentration in the solution by 2-3%, its sorption level is really not changed, while gemin sorption under the same conditions is increased by 7-10% which finally will not only increase protein yield, but will also reduce its gemin content. In fact, as the direct experiment has shown, upon addition of CMC to a 7% hemoglobin solution, the yield of lyophilically dried globin increased from 58 to 64% at practically the same content of iron in the final product (0.2%).

Electrophoretic study of the obtained globin was conducted on the device for vertical electrophoresis in the gel block 0.8 mm thick with the 10 x 10 cm size in tris-glycine byffer at the 30 mA-amperage and 210 V voltage during 2.5 hrs. Electrophoregram scanning was conducted on the "Hirschmann" densitometer (Germany).

As it is evident from the densitogram (Fig. 3), in the result of separation of the hem base from hemoglobin, practically homogeneous protein - globin was obtained.

The quality characteristics of globin, obtained in the result of CMC-bases separation of gemoglobin into hemin and globin are given in table 2. The scheme of separation of hemoglobin into hemin and globin is shown in Fig. 4.

References

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Table 1. Kinetic and sorption characteristics of the process of sorption of globin on carboxymethylcellulose

Table 2. Quality characteristics of globin

Fig. 1. Dependence of the hemin sorption value on its concentration in the solution.

Fig. 2 Dependence of the globin sorption value on its concentration in the solution.

Fig. 3. Densitogram of globin (M.M = 15 kD)

Fig. 4. Scheme of separation of hemoglobin into hemin and globin.