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IMMOBILIZATION OF MYOCARDIAL TROPOMYOSIN

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Tropomyosin (TM) is a regulatory myofibrillary protein with mol. wt. of 68,000-70,000 daltons [5]. Many workers have demonstrated its role in autoimmunity in patients with rheumatic diseases [3-7]. It was accordingly decided to study the isolation of TM and its use as an antigen in immunologic analysis. Immobilization of biologically active substances in the structure of a carrier polymer is a method of obtaining preparations that are resistant to various biophysical and biochemical action, with a long storage life [1].

The aim of this investigation was to create a granulated immobilized preparation of TM with a long keeping life and to study its properties and possible fields of application.

EXPERIMENTAL METHOD

TM was isolated from the cadaveric heart muscle of the clinically healthy person who had died as a result of an accident, and obtained not later than 8-10 h after death, by Bailey's method, followed by isoelectric reprecipitation at pH 4.3 [2]. The protein concentration was determined by Lowry's method [10]. The purified myocardial TM was incorporated into a polyacrylamide gel (PAAG) space lattice by emulsion polymerization [9]. The number of protein molecules incorporated into the space of the lattice of the gel and the rate of diffusion of the immobilized TM were monitored by the use of radioactive indicators. Radioactive labeling was carried out as in [8]. Since immobilized granulated myocardial TM with magnetic properties can be

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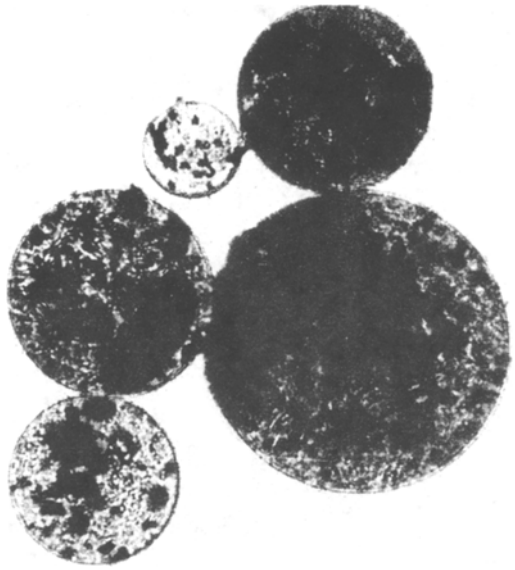

Fig. 1. Immobilized granulated myocardial TM with incorporated magnetic material (magnification 56).

used subsequently in immunologic reactions, we studied properties of this preparation under conditions in which this is most likely to be done. The effect of temperature, time, the high hydrogen ion concentration, and potassium thiocyanate on the rate of diffusion of [^{3}H]-TM from the PAAG space lattice was studied.

EXPERIMENTAL RESULTS

Immobilized cardiac TM of regular spherical shape, with incorporated magnetic material and with a particle size of 10-100 μm was obtained by emulsion polymerization (Fig. 1).

To obtain granules, a solution of [^{3}H]-TM was used. Immobilized granulated [^{3}H]-TM was obtained from 2.0 ml of solution with a protein concentration of 2.33 mg/ml. The specific radioactivity of the preparation was 32,752 Bq/mg protein, whereas the total radioactivity in the whole volume was 152,842 Bq. At the end of polymerization the preparation was washed to remove the catalyst, polymerization initiator, and emulsifier, with cold acetone and buffered physiological saline 3-5 times. Total activity in the washing fluid was 16,015 Bq, corresponding to 0.4896 mg protein. The percentage incorporation of myocardial TM, taken in a concentration of 2.33 mg/ml, and used to obtain granules was 89.5 (2.085 mg/ml), and when the labeled protein was present in a concentration of 3.5 mg/ml, the percentage fixation of the labeled TM was 69.3 (2.24 mg/ml). Despite this, incorporation of the absolute quantity of protein into the granules was about equal. Evidently during the use of 20% PAAG with a high concentration of the cross-linking agent methylene-*bis*-acrylamide, individual choice of the protein content during immobilization is essential for optimal preservation of its properties.

One of the main aims of this investigation was to create immobilized preparations of TM with a long keeping life, and accordingly diffusion of labeled TM was studied depending on time and temperature. The results (Fig. 2) indicate that most protein is removed from the granules on the 2nd-3rd day, after which this process takes place much more slowly. The total quantity of [^{3}H]-TM left in the granules after keeping for 6 days was 96.83%. On keeping for 30 days or more, there was a very small additional loss of 0.098%. The release of protein from the granules as a result of exposure to temperatures of +4, 20, and 37°C, with 3 M potassium thiocyanate solution and buffer at pH 2.2 (these solutions are often used to dissociate the antigen—antibody complex) is illustrated in Fig. 3a, b. Real losses of protein at the maximal temperature were $1.1 \cdot 10^{-5}$ mg/ml or 0.093%, and they can be disregarded.

The experiment showed that protein molecules, partly incorporated into the space lattice of the gel are present on the surface of the PAAG granules; during keeping, or exposure to various temperatures and a high H^+ concentration, the PAAG swells a little, and this leads to loss of some of them.

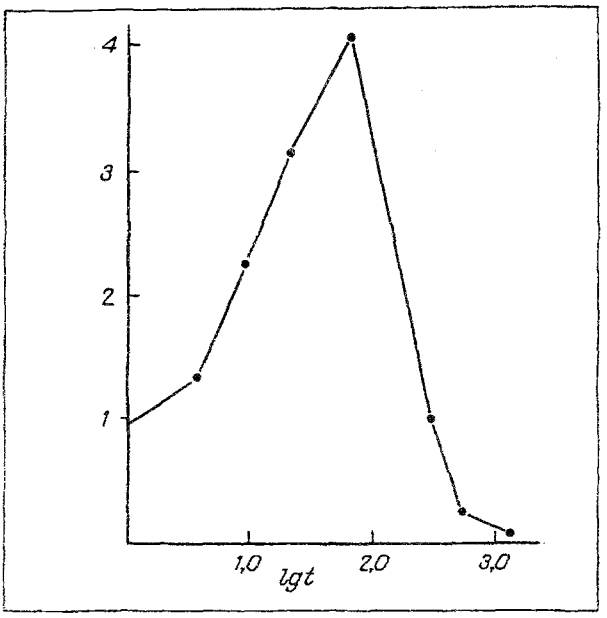

Fig. 2. Diffusion of [^{3}H]-TM from 20% PAAG granules depending on keeping time. Abscissa, log t; ordinate, protein concentration (in mg $\times$ 10^{-4}).

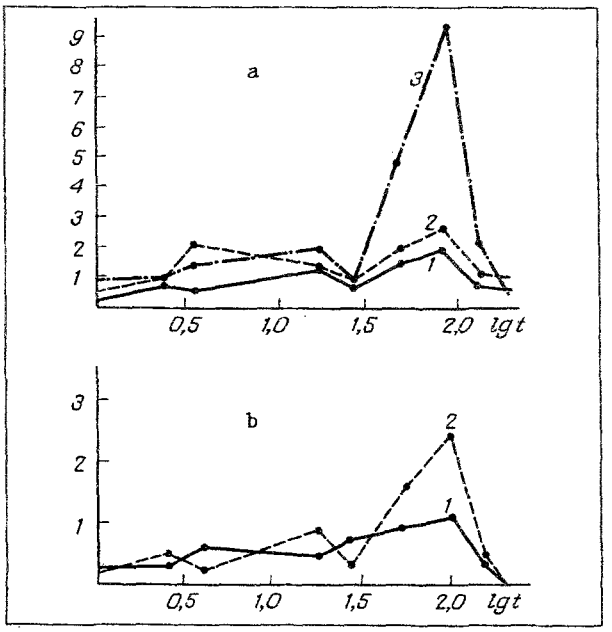

Fig. 3. Diffusion of [^{3}H]-TM from 20% PAAG: a) depending on temperature and time: 1, 2, 3) at 4, 20, and 37°C, respectively; b) depending on action of dissociating reagents and time: 1) 3 M solution of potassium thiocyanate (KCNS), 2) glycine-HCl (pH 2.2).

On treatment of the granules with 3 M potassium thiocyanate (Fig. 2b) no peak of TM elution was found, probably due to the action of a highly concentrated salt solution on the polyacrylamide granules, which become dehydrated, and this prevents removal of the protein molecules form the PAAG (like the action of a hypertonic solution on blood cells).

The possibility of reuse is confirmed by using the preparation in the immunofluorescence method of analysis of sera from rheumatic fever patients. The intensity of luminescence (in mV) of TM granules treated with sera from healthy individuals and patients with rheumatic fever was studied with the FMEL-1A instrument and SHCH-4300 digital voltmeter. The following

results were obtained: in healthy subjects (n = 12) 27.62 ± 4.12 mV, in patients with rheumatic fever in the active phase (activity 1, n = 23) 83.74 ± 9.95 mV ($p < 0.001$). Similar results were obtained on regenerated TM granules with these same groups of sera.

When immobilized TM was used as antigenic immunosorbent, pure antibodies to TM could be obtained. In model experiments to study the receptor function of immobilized biopolymers, preliminary treatment of the preparations at 37°C, with 0.1 M glycine-HCl buffer, pH 2.2, for 48 h was necessary to remove even the smallest trace of partially fixed biological material. The experimental data are evidence that immobilized granulated TM can be kept for 1 year at 4°C with the addition of preservatives (0.01% formalin or 1:5000 thiomersal). Under these circumstances diffusion of protein molecules from granules into solution did not take place. The interest in this preparation is due to the fact that it can be used as an antigen in order to detect specific antibodies in enzyme immunoassay and radioimmunoassay methods. The universality of this application can be attributed to the new properties which TM acquires due to its immobilization, namely resistance to biophysical and biochemical agents and invariable preservation of its immunologic properties. Incorporation of magnetic material into the immobilized TM simplifies manipulations with it and provides the basis for creation of automated diagnostic systems.

Thus immobilization in PAAG with a high concentration of a cross-linking agent, and with the incorporation of iron oxide yields stable preparations of TM of regular spherical shape with magnetic properties, a long keeping life, and frequent reuse in methods of preparative biochemistry and immunodiagnosis.

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