PRODUCTION OF MONOSPECIFIC ANTIBODIES TO HUMAN FERRITIN BY AFFINITY CHROMATOGRAPHY

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The sensitivity of the immunoradiometric method of determining serum ferritin, and also the reliability of the results, rise considerably when antibodies with a high affinity and a low cross-reactivity are used. In the present paper we describe an affinity method for obtaining monospecific antibodies to human ferritin which permits the isolation from the appropriate sera of native antibodies to ferritin with a high immunoreactivity.

Electrophoretically homogeneous ferritin [1, 2] was used to immunize rabbits with the aim of obtaining monovalent antisera to this protein. Immunization was performed by a scheme including four subcutaneous injections of ferritin with complete Freund's adjuvant with a two-weak interval between injections. The total amount of antigen used for primary immunization was 4 mg. On the 1st day after the last injection blood was taken from the auricular vein of the rabbit in an amount of 30 ml and it was kept in the refrigerator at +4°C for 12 h. The antiserum was drawn off and was stored at −20°C until used. The antibody titer in the antiserum according to double radial immunodiffusion in agar was 1:64.

To isolate the antibodies from the antiserum a specific sorbent was prepared which consisted of ferritin covalently conjugated with cyanogen-bromide-activated Sepharose 4B (Sweden). To prepare the sorbent, ferritin and Sepharose were taken in initial amounts of 5 mg and 1.5 g, respectively. The immobilization of the protein on the activated Sepharose was performed in a 0.3 M solution of $NaHCO_3$ with gentle stirring by a magnetic stirrer at room temperature for 24 h. After this, the first fraction was transferred to a 0.7 × 10 cm column which was washed with large portions of a 0.3 M solution of sodium bicarbonate. In order to block the unoccupied active sites of the sorbent, a 0.2 M solution of glycine, pH 8.0, was passed through the column. Then it was again washed with large portions of 0.3 M $NaHCO_3$ and with sodium phosphate buffer, 0.1 M, pH 8.0, containing 0.5 M NaCl. The column prepared in this way was used to isolate the monospecific antibodies from the antiserum, for which purpose the antiserum was passed through the column at the minimum rate of flow. After the sorption of the antibodies, the column was washed with 0.1 M sodium phosphate buffer, pH 8.0, containing 0.5 M NaCl. The antibodies were eluted with 0.1 M sodium acetate buffer, pH 2.9, containing 1 M NaCl (Fig. 1). The protein in the eluate was recorded from its absorption at 280 nm with the aid of a Uvicord II (Sweden). The pH in the antibody fraction was brought to 7.2 with a 0.5 M solution of NaOH, after which the antibodies were desalted on a column of Sephadex G-25, and were concentrated and stored in the frozen state.

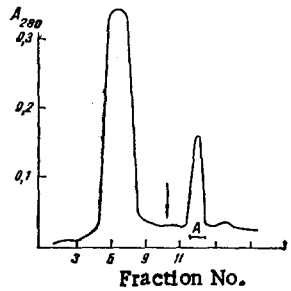

Fig. 1. Elution profile of antiserum to human ferritin on a column of ferritin-Sepharose sorbent (the arrow shows the beginning of elution with 0.1 M sodium acetate buffer, pH 2.9, containing 1 M NaCl); A) fractions containing antibodies to ferritin.

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The native state of the antibodies to ferritin obtained by affinity chromatography was checked by immunoelectrophoresis, which gave only one precipitation arc with the antigen.

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RAPID AUTOMATED SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES USING A NEW CONDENSING REAGENT

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Continuing investigations in the field of the solid-phase synthesis of oligonucleotides and the preparation of artificial DNA fragments from them [1-4], we have considered the possibility of a rapid automated synthesis of oligodeoxyribonucleotides using a new condensing reagent — a mixture of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) and 4-dimethylaminopyridine 1-oxide (DMAPO), the efficacy of which has been demonstrated in the synthesis of oligonucleotides in solution.

The solid-phase synthesis of undecathymidylate and of three oligomers with a length of 9-11 units was performed on a "Viktoriya-2" automatic apparatus with a modified hydraulic system. As the polymeric support we used one based on Silochrom silica gel C-80 [3]. For chain growth we used a 0.1-0.15 M solution of protected mono-, di-, and trinucleotide blocks in absolute pyridine activated with a mixture of TPS (3 equivalents) and DMAPO (7 equivalents). The excess of the nucleotide component calculated on the first nucleoside was 5-6 equivalents. The sequence of washings of the support was similar to that described previously [4] but their total duration was decreased to 22 minutes. The time of condensation with the mononucleotides amounted to 2-3 min, and with the di- and trinucleotide blocks to 5-6 min, so that one cycle of chain growth on the support did not exceed 30 min, regardless of the length of the nucleotide block. The average yield per condensation stage determined from the dimethoxytrityl carbinol liberated [3] was 80-86%. After detachment from the support and complete deblocking of the reaction mixtures by the action of concentrated ammonia (at 45°C for 16 h), the following were isolated by high-performance liquid chromatography under the conditions described in [3]: 28.4% of d(TTTTTTTTTT), 17.7% of d(GATCTCAGC), 22% of d(GCGTTCCTTC), and 11.5% of d(AATTGGATCAT). The sequences of the nucleotides in the oligomers synthesized were confirmed by the Maxam-Gilbert method.

Thus, in the present work it has been shown that the use of the new condensing agent — a mixture of TPS and DMAPO — leads to a considerable acceleration of the condensation stage in the solid-phase synthesis of oligodeoxyribonucleotides and permits the desired oligomers to be obtained rapidly in high yield.

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