Dilated cardiomyopathy is one of the main reasons of heavy cardiac decompensation and the most frequent cause of cardiac death [1]. Clinical evidence demonstrates that procedures of therapeutic apheresis of blood plasma of patients with DCMP [3–5] are most effective in comparison with those containing linear peptide precursors. They demonstrate that the sorbents on the basis of the conformational antigens were more effective in comparison with those containing linear peptide precursors.

Abstract—Two fragments corresponding to the 125–133 and 206–218 sequences of a molecule of the β1-adrenoreceptor (autoantibodies to this protein are often found in patients with dilated cardiomyopathy) were synthesized by the solid phase method with the use of Fmoc technology. Two new conformational antigens were prepared by directed (regioselective) and undirected (spontaneous) formation of intramolecular and intermolecular disulfide bridges between the corresponding cysteine residues of the synthesized peptides. One of these antigens consisted of a mixture of disulfide isomers, and another antigen was an isomer with a natural arrangement of S–S bridges. Immunosorbents were obtained by immobilization of the synthesized antigens on the bromocyanogen-activated sepharose and applied to the removal of autoantibodies in a β1-adrenoreceptor from the blood plasma of patients. We demonstrated that the sorbents on the basis of the conformational antigens were more effective in comparison with those containing linear peptide precursors.

Key words: peptide antigens, spontaneous and regioselective formation of disulfide bonds, β1-adrenoreceptor, dilated cardiomyopathy.

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INTRODUCTION

Dilated cardiomyopathy is one of the main reasons of heavy cardiac decompensation and the most frequent cause of heart transplantation. Pathogenesis of this disease is not studied in detail. A hypotheses of chronic virus infection and genetic determination are proposed, but recent published data point to an autoimmune character of this heavy pathology [1, 2]. Clinical evidence demonstrates that procedures of therapeutic apheresis of immunoglobulins considerably improve the state of patients with patients with DCMP [3–5]. Autoantibodies that are found in the serum of DCMP patients mainly belong to the immunoglobulins of G class (IgG) and are antibod-

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2 Abbreviations: Acm, acetamidomethyl; DCMP, dilated cardiomyopathy; DIC, N,N'-diisopropylcarbodiimide; Fmoc, 9-fluorenlymethyloxycarbonyl; HOBt, 1-hydroxybenzotrazole; NMP, N,N'-methylpyrrolidone; TIBS, trisobutylsilane; Pmc, 2,2,5,7,8-pentamethylchromane-6-sulfonyl; SPyr, 2-pyridinesulfonyl; SPPS, solid phase peptide synthesis. Other abbreviations correspond to IUPAC-IUB: Eur. J. Biochem, 1994, vol. 183, pp. 9–37.

of a β1-adrenoreceptor are most often found in patients with DCMP [7].

The introduction of the peptide corresponding to the sequence of the second loop of a β1-adrenoreceptor in rabbits has been shown to result in DCMP development [8]. Hence, the presence of autoantibodies of this specificity also plays a key role in pathogenesis of this disease. Moreover, the presence of antibodies in a β1-adrenoreceptor has been found to be one of the factors of the development of tachyarrythmia and acute cardiac decompensation [9, 10].

β1-Adrenoreceptor has three extracellular and three intracellular polypeptide sequences (loops) with seven transmembrane α-helical domains. Two disulfide bridges (Cys209–Cys215 in the second loop and Cys133–Cys136 between the first and the second loop) are in the molecule (Fig. 1) [11, 12].

Peptide fragments of the second extracellular loop of a β1-adrenoreceptor are known to be antigenic determinants which can be bound to autoantibodies in this protein [7, 13].
of fragments of the first and the second extracellular loops (the Glu-Tyr-Gly-Ser-Phe-Phe-Cys-Glu-Leu nonapeptide and Ala-Arg-Arg-Cys-Tyr-Asn-Asp-Pro-Lys-215Cys-218Phe tridecapeptide corresponding to the 125–133 and 206–218 sequences of the β1-adrenoreceptor, respectively [3]) immobilized on the bromocyanogen-activated agarose matrix. From our point of view, the Coraffin® columns have a significant disadvantage. Each of the two sorbents in the mechanical mixture contains one of the aforementioned peptides and binds autoantibodies specific to linear antigenic determinants which are located only in the first or only in the second extracellular loop of a β1-adrenoreceptor.

As it has been mentioned above, two disulfide bonds are present in the native molecule. Disulfide bonds are known to have various functions in proteins. They stabilize folded proteins. The thiol–disulfide exchange can be a basis for regulation of enzymatic activity, and so forth. Moreover, both cysteine residues of the Cys215-Cys216 sequence, which is located in the second extracellular loop of a β1-adrenoreceptor, participate in the formation of S–S bridges. This Cys–Cys sequence is an important structural element of a number of proteins [14]. It creates the possibility of binding and approximation of three segments of the amino acid chain.

According to the published data, the disulfide bond between the Cys131 and Cys216 residues plays an important role in supporting the conformation of the ligand-binding site of the protein [15]. The presence of disulfide bridges in a molecule of a β1-adrenoreceptor most probably contributes to the antigenic properties of this protein (the existence of a conformational antigenic determinant to which autoantibodies can form is quite possible). Thus, the affinity of such a sorbent as Coraffin® will be inevitably insufficient for removal of all autoantibodies to the β1-adrenoreceptor. This conclusion was experimentally proven [3] and could possibly explain the fact that no new data on the application of