SUMMARY

!. A new polymeric sorbent for proteinases has been synthesized by the radical copolymerization of N-vinylpyrrolidone, bis-N^{δ}-acryloylgramicidin-C, and N,N'-methylenebisacrylamide.

2. Biospecific chromatography on the new sorbent has permitted the 2.5-fold purification of an industrial preparation of porcine pepsin.

3. With the aid of the new sorbent, a carboxylic proteinase has been isolated from the industrial preparation Tsellolignorin at a purification factor of 15.

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SPIN-LABEL STUDY OF THE CONFORMATIONAL STATES OF POLYPEPTIDE MODELS OF HISTONES

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UDC 541.65+547.466.1

The conformational states of the regular polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$, $(Ala\text{-}Orn\text{-}Gly)_n$, and $(Ala\text{-}Orn\text{-}Ala)_n$ have been studied by the spin-label method. Their behavior in solutions of guanidine hydrochloride and urea and in solutions of salts of bivalent metals does not contradict the presence of an extended levohelical conformation in their polypeptide chains. In $CaCl_2$ (5 N) solutions the polypeptides exhibit aggregation properties. A study of the behavior of the poly peptide at these temperatures has shown that with a rise in temperature there is a monotonic change in the structures of the polypeptide chains that is characteristic for a conformation of the polyproline-II type. Differences have been observed in the behavior of glycine- and alanine-containing polypeptides in the presence of sodium dodecyl sulfate with a change in the temperature.

To understand the molecular mechanisms of the interaction of histones with DNA in the formation of DNP complexes it is important to know the potential structural possibilities of the polypeptide chains of the histones participating in this interaction. According to modern ideas on the structure of chromatin [1, 2], such sections in histones are the N- and C-terminal segments which are enriched with basic amino acids. One of the approaches to the study of the conformation of features of the terminal sections of histones is the use of model polypeptides of regular structure with given sequences of amino acid residues.

With this aim, we have synthesized the polypeptides $(Gly\text{-}Lys\text{-}Gly)_n$, $(Gly\text{-}Orn\text{-}Gly)_n$, $(Ala\text{-}Orn\text{-}Gly)_n$, and $(Ala\text{-}Orn\text{-}Ala)_n$ [3, 4] modeling the N-terminal sections of bovine histones enriched with glycine and basic amino acid residues, and the C-terminal sections of histones H1 enriched with alanine and basic amino acid residues [5-7].

The conformational states of the regular polypeptides synthesized were investigated by the spin-label method. This method has found wide use in the solution of such problems as

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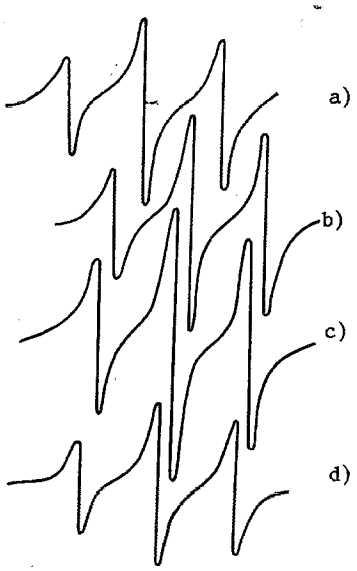

Fig. 1

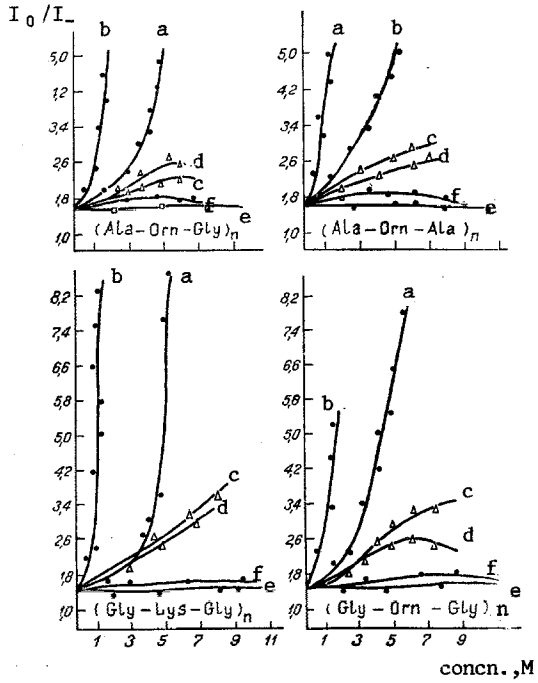

Fig. 2

Fig. 1. ESR spectra of the spin-labeled polypeptide: a) (Gly-Lys-Gly)$_n$; b) (Gly-Orn-Gly)$_n$; c) (Ala-Orn-Gly)$_n$, I_0/I_- = 1.88, 1.85, 1.82, and 1.87, respectively; C_n = 1 mg/ml; T = 24°C.

Fig. 2. Change in the parameters of the rotational diffusion of the spin labels in the polypeptides, I_0/I_- as a function of the concentrations of various reagents; a) sucrose; b) $CaCl_2$; c) $MgCl_2$; d) $ZnCl_2$; e) guanidine hydrochloride; f) urea; C_n = 1 mg/ml; T = 24°C.

recording the conformational changes of macromolecules under the perturbing actions of a medium, the study of the mechanism of the action of enzymes and of the structures of their active sites, the determination of the dimensions of protein macromolecules in solution, and the study of the dynamic characteristics of individual sections of biological materials [8, 9].

Spin-label polypeptides were obtained by a procedure that we have developed [10]. The fact that the modification of the polypeptides investigated by the spin label took place at a pH of above 8.5 apparently indicates that nucleophilic substitution took place at lysine ε-NH_2 groups. Under these conditions, a reaction with the α-NH_2 groups (pK 7) takes place at a considerably lower rate.

No differences were found between the spin-labeled and the unlabeled polypeptides by gel filtration and spectrophotometry, which indicates that the modification of the lysine and ornithine residues did not appreciably affect the conformations of the polypeptides. The ESR spectra of the spin-labeled polypeptides are given in Fig. 1. The form of the ESR spectra indicates a rapid rotation of the radical fragment. The concentrations of the spin labels attached to the polypeptides were determined with an accuracy of 5% by a quantitative comparison of the ESR spectra of the spin-labeled polypeptides with the spectra of standard labels in aqueous glycerol at 77°K [8]. The loading factor of the spin labels, i.e., the number of labels per polypeptide molecule was between 0.98 and 1.08.

It is known that such detergents as urea, guanidine hydrochloride, and sodium dodecyl sulfate possess the capacity for unfolding the polypeptide chains of macromolecules. The capacity of metal ions for affecting mutual orientation of the different sections of the molecules of proteins or other macromolecules is also known. The binding of a metal ion may cause radical changes in the conformations of molecules. In view of this, we studied the structural behavior of the regular spin-labeled polypeptides in solutions containing urea, guanidine hydrochloride, sodium dodecyl sulfate (Na-DDS) and bivalent metal ions in the form of $ZnCl_2$, $MgCl_2$, and $CaCl_2$.

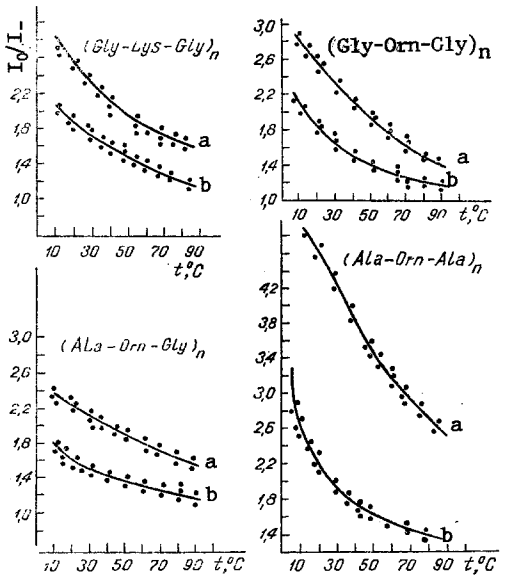

Fig. 3. Change in the parameters of rotational diffusion of the spin labels in the polypeptides, I_0/I_-, as a function of the temperature in the presence of a 1% solution of sodium dodecyl sulfate (a) and without it (b); C_n = 1 mg/ml.

Figure 2 gives curves of the change in the parameters of rotational diffusion of the spin labels in the polypeptides (I_0/I_-) as a function of the concentration of the added reagents. The change in the parameter ratio I_0/I_- characterizes a more rigid or more flexible conformation of the protein in the region of the added spin labels as a result of the corresponding action.

Usually, under the action of urea and guanidine hydrochloride on the structure of spin-labeled protein molecules there is a sharp freeing of the rotation of the labels recorded by the ESR spectrum [8, 9, 11]. In the case of the labeled polypeptides, no such action was observed. As can be seen from Fig. 2e, f, the ESR of the spin-labeled polypeptides did not change appreciably on titration with urea and with guanidine hydrochloride. This indicates that under the action of such reagents no appreciable local conformational changes take place in the polypeptides in the region of attachment of the spin labels. This fact can be explained by the presence in the polypeptide chains of a comparatively stable conformation of an extended left-handed helix, which is stabilized by guanidine hydrochloride and by urea [12], in contrast to such regular structures as an α-helix and a β-structure. These results correlate with those obtained by the optical rotation and circular dichroism methods [13].

When the spin-labeled polypeptides were titrated with magnesium chloride and zinc chloride, appreciable changes took place in the ESR spectra (Fig. 2c, d) indicating some local structural rearrangements in them. Particularly sharp changes in the parameters of rotational diffusion of the spin labels in the polypeptides were observed when calcium chloride was added (Fig. 2a), which indicated considerable local conformational changes taking place in the polypeptide chain. Similar effects in the structures of polypeptides have also been detected by the circular dichroism method [14]. Apparently, such conformational rearrangements in a polypeptide chain take place as the result of an interaction of the Ca^{2+} ions with the carbonyls of the peptide groups [15]. Similar changes in the parameters of rotational diffusion of a spin label in a polypeptide have been detected only when the viscosity of the solution was changed substantially with the aid of sucrose, where an appreciable decrease in the "frequency of rotation" of the label in a polypeptide was also observed (Fig. 2b). The marked inhibition of the rotation of the radical possibly indicates a more pronounced aggregation of the polypeptide molecule in $CaCl_2$ solution than under other conditions.

The behaviors of all four spin-labeled polypeptides under the conditions studied were qualitatively similar.

The spin-labeled polypeptides were also investigated at various temperatures in the presence of Na-DDS and without it. The rotational mobility of a label in a polypeptide matrix changes with a rise in the temperature but not so sharply as is characteristic for histones and other biopolymers. An analysis of the parameters of rotational diffusion,

I_0/I_- of the spin-labeled polypeptides in the presence of Na-DDS and without it (Fig. 3a, b) showed that with a rise in the temperature there was an appreciable change in the ESR spectra. The change in the spectra indicated a freeing of the rotational motion of the radical apparently caused by conformational rearrangements in the polypeptides. The change bore a monotonic noncooperative nature. In all cases, in the presence of Na-DDS the ESR spectra showed that the spin labels in the polypeptides were more immobilized than without the Na-DDS, while for the polypeptide $(Ala\text{-}Orn\text{-}Ala)_n$ the freeing of the rotation of the radical with a change in the temperature had a more pronounced nature than for the other polypeptide as the result of a destabilization of its α-helical conformations. The existence of such destabilization with a rise in the temperature has also been shown by ORD spectra [13].

Consequently, we have observed differences in the behaviors of two different types of polypeptides (glycine-containing and alanine-containing) which consist in the fact that, while in the presence of Na-DDS a lowering of the temperature stabilizes the extended left-handed helical conformation existing in aqueous solutions of glycine-containing polypeptides, for alanine-containing polypeptides a lowering of the temperature leads to a stabilization of the α-helical conformation [13]. All the temperature changes of the ESR spectra are reversible.

EXPERIMENTAL

To modify the polypeptides we used the spin label 3-amino-2,4-dichlorotriazino-2,2,5,5-tetramethylpyrrolidine-1-oxyl [sic]. All the ESR spectra were taken on RÉ-1301 and RÉ-1306 radiospectrometers (USSR) in glass tubes of the same diameter (1 mm) containing the same amounts of solutions. The temperature dependences of the spectra of the spin-labeled polypeptides were obtained on the RÉ-1306 with a thermostated resonator.

To determine the dependence of the parameters of the ESR spectra on the concentrations of urea, guanidine hydrochloride, sucrose, $CaCl_2$, $ZnCl_2$, and $MgCl_2$, aqueous solutions of spin-labeled polypeptides with the same concentration (1 mg/ml) and different amounts of the above-mentioned reagents were prepared.

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SUMMARY

The conformational states of spin-labeled polypeptides modeling the terminal sections of various histones enriched with basic amino acids residues in combination with alanine and glycine residues have been investigated. Their behavior in solutions of guanidine hydrochloride, and of urea, and in solutions of salts of bivalent metals does not contradict the presence of extended levohelical conformations in their polypeptide chains. At the same time, alanine-containing polypeptides adopt a partially α-helical conformation in the presence of Na-DDS. In a calcium chloride solution (5 M) the polypeptides exhibit aggregation properties. A study of the polypeptides at different temperature showed that with a rise in the temperature there was a monotonic change in the structures of the polypeptide chains that is characteristic for a conformation of the polyproline-II type.

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SYNTHESIS OF THYROLIBERIN

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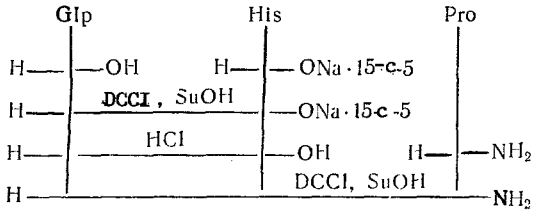
A two-stage method for the synthesis of thyroliberin is proposed. A complex of the sodium salt of histidine with 15-crown-5 is used for the protection of the carboxylic fraction of histidine.

Since the time of the isolation of thyroliberin (TRH) – a hormone of the hypothalamus – in 1969 [1], and the discovery of its antidepressant properties [2], several multistage methods for its preparation from protected amino acids have been published [3].

We have performed a two-stage synthesis of TRH with the minimum use of protective groups by the following scheme*



The condensation was performed by the carbodiimide method in dimethylformamide in the presence of N-hydroxysuccinimide. The use of a 1.1-molar excess of N-hydroxysuccinimide substantially suppressed racemization [4]. The carboxylic function of histidine was protected by salt-formation. An aqueous solution of sodium salt of histidine was mixed with an equivalent amount of 15-crown-5 [5]† and dimethylformamide, and then the water and 2-3 ml of the dimethylformamide were driven off in vacuum and the residue was treated with a mixture of pyroglutamic acid, N-hydroxysuccinimide, and N,N'-dicyclohecylcarbodiimide in dimethylformamide. The sodium salt of pyroglutamylhistidine was decomposed with an equivalent amount of HCl solution.

The pyroglutamylhistidine and the thyroliberin were purified by column chromatography on silica gel. The physicochemical characteristics of the thyroliberin obtained corresponded to those given in the literature [1].

EXPERIMENTAL

The purity of the compounds obtained was monitored by the TLC method on Silufol plates (Kavalier, Czechoslovakia). Reanal (Hungary) L-amino acids were used. Amino acid analysis was performed on a Hitachi amino acid analyzer. The amino acid and elementary analyses corresponded to the compositions of the peptides aimed at.

* Abbreviations: DDCI) N,N'-dicyclohexylcarbodiimide; SuOH) N-hydroxysuccinimide; Glp) pyroglutamic acid; His) histidine; Pro) proline.
† The nomenclature of the crown ethers has been taken from [5].

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