

CONFORMATIONAL-TRANSITIONS OF BOVINE PANCREATIC RIBONUCLEASE S-PEPTIDE *

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1 Introduction

Several authors have recently studied RNase A [1-4] and RNase S [3] by ORD and CD techniques. The results obtained show that the main structural features of both molecules, in aqueous solution, are closely related and consistent with the structures elucidated by X-ray studies [5, 6]. The crystallographic data show that in both molecules about 50% of the N-terminal eicosapeptide is in an α -helical conformation [5, 6]. However, in a previous communication [7] we showed that the S-peptide, in aqueous solution, is essentially in random form when separated from the S-protein.

On the basis of these results it was proposed [7] that in the binding process the S-peptide undergoes a coil-to-helix transition.

The aim of the present paper is to give experimental evidence for the preceding hypothesis by demonstrating that the S-peptide is indeed able to assume a helical structure through a solvent-induced conformational transition.

In fact, as already suggested [7], one can assume that the presence of the S-protein is equivalent to a change in the solvent composition which is able to induce in the S-peptide a conformational transition

towards helix formation by a decrease of the dielectric constant of the medium.

Accordingly we have investigated by circular dichroism the S-peptide conformation in the presence of SDS and TFE, which are known to cause a transition from the random to the helical form in polypeptide systems.

2. Experimental procedures

S-Peptide was prepared according to the literature [8-10]. TFE was obtained from Halocarbon Products (Hackensack, N. J.) and used without further purification. Recrystallised SDS was obtained from Schuchardt (München). CD measurements were made with a Jouan Mod. CD 185 dichrograph, with nitrogen-flushing below 200 nm. The instrument sensitivity was kept at 1 to 2×10^{-5} . Cylindrical fused quartz cells with 0.1 and 0.05 cm path lengths were used. The usual instrumental precautions were taken to avoid artefacts. All solution measurements were performed at peptide concentrations in the range 0.3-1.0 mg/ml. Twice distilled water was used. The data are expressed in terms of $[\theta]_{\lambda}$, the mean residue molecular ellipticity, defined as $[\theta]_{\lambda} = 3300 (\epsilon_l - \epsilon_r)$, $\text{deg cm}^2 \text{ dmole}^{-1}$, where $(\epsilon_l - \epsilon_r)$ is the difference between the molar (on a mean residue basis) extinction coefficients for left and right circularly polarized light.

* For the nomenclature regarding S-peptide, S-protein, RNase A, and RNase S see F. M. Richards, Proc. Natl. Acad. Sci. US 44 (1958) 162. Abbreviations used: ORD, optical rotatory dispersion; CD, circular dichroism; SDS, sodium dodecyl sulphate; TFE, trifluoroethanol.

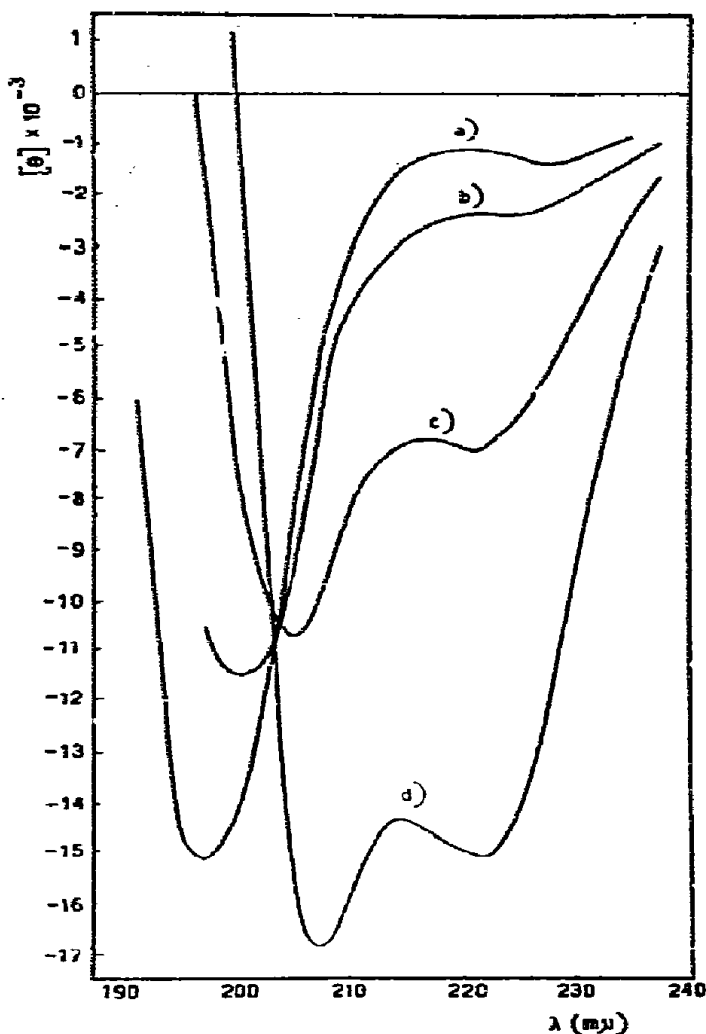


Fig. 1. Far-ultraviolet CD of S-peptide: (a) in water; (b) in 0.05 M SDS; (c) in water-TFE (1:1); (d) in 100% TFE.

3. Results and discussion

In fig. 1 the CD spectra of the S-peptide in water, 0.05 M SDS, water-TFE (1:1), and 100% TFE are reported. As is clearly discernible, the negative band originally present at 227 nm in water is shifted to 225 nm in 0.05 M SDS and to 222 nm (the typical wavelength of the amide $n-\pi^*$ transition of the α -helix [11]) in the presence of TFE. Furthermore the rotational strength of the band is enhanced in solutions of increasing TFE concentration (figs. 1 and 2). Correspondingly, the large negative band centered on ca.

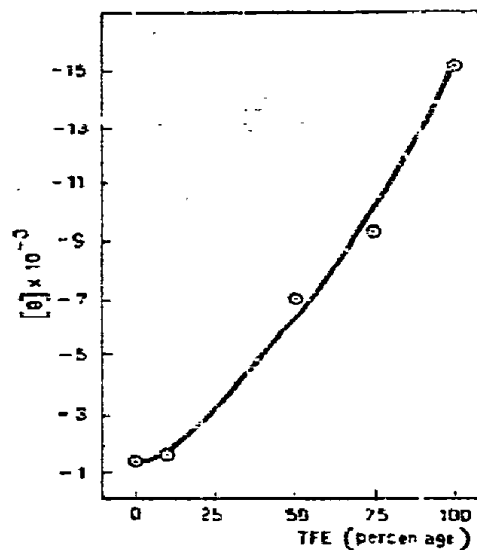


Fig. 2. Solvent-induced conformational transition of S-peptide. Solutions prepared by mixing S-peptide in water and TFE.

198 nm in water and associated with the amide $\pi-\pi^*$ transition of disordered polypeptides [11], vanishes and is replaced by another negative band which, in 100% TFE, appears at 207–208 nm, i.e. at the typical wavelength of the amide parallel $\pi-\pi^*$ transition of the α -helix [11].

It is evident that the S-peptide becomes helical by undergoing a solvent-induced conformational transition and that the value of approx. 55% of α -helix in 100% TFE is reached.

It is interesting to recall that, according to X-ray data, the S-peptide is 50% helical when associated to the S-protein [6]. Fig. 2 shows the variation of the mean residue molecular ellipticity $[\theta]$ at the maximum of the longer-wavelength band as a function of the solvent composition. The curve obtained is very similar to that exhibited by glucagon in water-2-chloroethanol mixtures [12]. In addition glucagon has been shown to form α -helices in alkaline aggregation [13, 14] and presumably also on crystallisation [15]. This behaviour seems to be paralleled by the S-peptide in its non-covalent association with S-protein. In both cases the transition would be energetically favoured by the greater extent of the hydrophobic interactions.

It will be of interest to see whether the property of the S-peptide to undergo a solvent-induced transition also extends to the synthetic S-peptide analogues

which we have prepared [16], and to correlate such properties with their S-protein activating capacities. Finally the following speculations may be made. The role of the S-peptide in the generation of ribonuclease activity, when S-peptide and S-protein unite, has been related by Hofmann [17] and by Finn and Hofmann [18] to the mode of action of the polypeptide hormones by assuming that the hormones transform cell receptor sites into enzymes. We think that, analogously to the S-peptide, the linear polypeptide hormones, owing to their relatively low molecular weights, generally possess in solution a variety of conformations which are continuously interchanging, that is to say, they are molecules with some degree of flexibility. By interacting with the receptor the hormone is forced into the proper conformation to constitute the active enzyme.

Furthermore, as the S-peptide constitutes the N-terminal part of the intact protein, the results given in the present paper are of interest in connection with the problem of the conformation assumed by the growing polypeptide chain as it comes off the ribosome. Studies along this line are in progress.

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