CONFORMATIONAL TRANSITION OF THE SYNTHETIC 2–15 *N*-TERMINAL FRAGMENT OF HEN EGG-WHITE LYSOZYME†

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1. Introduction

It has been proposed [1] that the folding of a protein molecule begins at the amino end even before the synthesis is complete. Such a sequential mechanism presumes that the protein conformation is primarily due to interactions between amino acid residues close in the sequence and implies that parts of the polypeptide chain, particularly those near the terminal amino end, may fold into stable conformations that can still be recognized in the complete molecule and act as 'internal templates' around which the rest of the chain is folded.

The results obtained by studying the conformational properties of the *N*-terminal eicosapeptide (S-peptide) [2, 3] of bovine pancreatic ribonuclease and of several S-peptide synthetic analogs [4] in aqueous solution and in the presence of helicogenic solvent, do not agree with the hypothetical sequential mechanism mentioned above. The hen egg-white lysozyme was shown by X-ray analysis [5] to contain three runs of helix (residues 5–15, 24–34 and 88–96) for which the axial translation per residue and the number of residues per turn fall close to the α-helix values.

The aim of the present paper is to report some preliminary conformational studies on the synthetic tetradecapeptide corresponding to the 2–15 *N*-terminal fragment of hen egg-white lysozyme. The peptide conformation has been investigated by circular dichroism in aqueous solution and in the presence of trifluoroethanol which is known to cause a transition from the random to the helical form in polypeptide systems.

2. Experimental procedure*

The tetradecapeptide valylphenylalanylglycylarginyl-*S*-acetamidomethylcysteinylglutamylleucylalanylalanylalanylmethionyllysylarginylhistidine methyl ester has been synthesized by condensation of three fragments corresponding to the sequences 2–4, 5–11 and 12–15 which have been prepared by stepwise elongation starting from the *C*-terminal amino acid.

The thiol group of cysteine in position 6, which in the native molecule forms a disulfide bond with the residue in position 127, was present as *p*-methoxybenzyl derivative [6] during the synthesis and as *S*-acetoamido methyl derivative [7] in the final product.

In the stepwise elongation, the activation of the carboxyl group was achieved by the active ester method with the exception of the valyl residue which was introduced, as benzyloxycarbonyl derivative, by the mixed anhydride procedure.

Whenever racemisation could occur, the condensations of the peptide subunits were carried out by an azide coupling step.

† Part 1.

* The peptides and peptide derivatives mentioned have the L-configuration. For a simpler description the customary L-designation for individual amino acid residues is omitted.

The following abbreviations are used [IUPAC-IUB Commission on Biochemical Nomenclature, European J. Biochem. 1 (1967) 375] Z = benzyloxycarbonyl; Boc = *tert*-butyloxycarbonyl; Nps = *o*-nitrophenylsulfenyl; Bz = benzyl ester; OMe = methyl ester; Mbz = *p*-methoxybenzyl; DMF = dimethylformamide; AP-M = aminopeptidase M; Acm = acetoamidomethyl.

The condensation of the tripeptide Z–Val–Phe–Gly-OH (fragment 2–4) ($[\alpha]_D^{25}$ −20.0 ± 0.5°, *c* 0.5, DMF; mp. 207–210°) obtained by saponification of the corresponding ethyl ester [8] with the heptapeptide H-Arg(NO_2)–Cys(MBz)–Glu(Bz)–Leu–Ala–Ala–Ala–NH–NH–Boc ($[\alpha]_D^{25}$ −17.0 ± 1°, *c* 0.58, DMF; mp. 237–239°) corresponding to fragment 5–11, was carried out by the dicyclohexylcarbodiimide-*N*-hydroxysuccinimide procedure [9].

The heptapeptide 5–11, free base, was obtained from the *N*-protected heptapeptide Nps–Arg(NO_2)–Cys(MBz)–Glu(Bz)–Leu–Ala–Ala–Ala–NH–NH–Boc ($[\alpha]_D^{25}$ −26.0 ± 1°; *c* 1.0, DMF; mp. 226–229°; amino acid ratios in acid hydrolysate* $Arg_{0.93}$, 1/2 $Cys_{0.96}$, $Leu_{1.00}$, $Ala_{3.04}$, $Glu_{1.06}$) by treatment with thioacetamide [10].

The protected decapeptide hydrazide 2–11 ($[\alpha]_D^{25}$ −27.2 ± 0.5°, *c* 0.74, DMF; mp. 264–267°; amino acid ratios in acid hydrolysate: $Arg_{0.95}$, 1/2 $Cys_{0.94}$, $Glu_{1.02}$, $Gly_{1.08}$, $Ala_{3.07}$, $Val_{1.03}$, $Leu_{1.00}$, $Phe_{0.95}$) was treated with trifluoroacetic acid to remove the *t*-butyloxycarbonyl protecting group from the *C*-terminal hydrazide and condensed, via an azide coupling step, with the tetrapeptide H-Met–Lys(Z)–Arg(NO_2)–His–OMe (fragment 12–15) ($[\alpha]_D^{25}$ + 9.4 ± 0.2°, *c* 0.93, DMF; mp. 155–160°). The latter was prepared from the corresponding N^{α}-*t*-butyloxycarbonyl crude derivative, by treatment with trifluoroacetic acid.

The resulting protected tetradecapeptide 2–15 was first treated with anhydrous hydrogen fluoride, in the presence of anisole [11] and methionine [12, 13] to remove all of the protecting groups, with the exception of the *C*-terminal methyl ester.

Subsequent treatment of this solution with hydroxymethylacetamide [14] protected the thiol group of cysteine in position 6 as acetamidomethyl derivative. After removal of the hydrogen fluoride, the crude material was dissolved in water, purified by chromatography on Amberlite IRC 50 using 0.2 M ammonium acetate with pH gradient as the eluent, and desalted on a small bed of BioRex.

The peptide was then eluted from the resin with aqueous acetic acid, lyophylized and incubated with aqueous thioglycolic acid to reduce any methionine sulfoxide (Amino acid ratios in acid hydrolysate*: $Arg_{1.98}$, $Lys_{0.96}$, $His_{0.95}$, $Glu_{1.10}$, $Gly_{0.96}$, $Ala_{3.10}$, $Val_{0.92}$, $Leu_{1.05}$, $Phe_{0.90}$, $Met_{0.85}$; amino acid ratios in AP-M digest [15]; $Arg_{1.90}$, $Lys_{0.91}$, $(His + His\,OMe)_{0.96}$, $Cys\,(Acm)_{0.98}$, $Glu_{1.09}$, $Gly_{1.00}$, $Ala_{2.90}$, $Val_{1.03}$; $Leu_{0.99}$, $Phe_{1.04}$, $Met_{1.05}$; single component on paper electrophoresis at pH 1.9, 6.4 and 9.4).

The details of the synthesis will be published elsewhere. AP-M was obtained from Rohm and Haas GmbH, Darmstadt, West Germany.

Trifluoroethanol was obtained from Halocarbon Products (Hackensock N.J.) and used without further purification. Circular dichroism (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 X 10. Cylindrical fused quartz cells with 1.0, 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 0.5–1.0 mg/ml. Twice distilled water was used. The data are expressed in terms of $[\theta]_\lambda$, the mean residue molecular ellipicity defined as $[\theta]_\lambda = 3300\ (\epsilon_l - \epsilon_r)$ deg cm^2 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.

3. Results and discussion

The circular dichroism curves of the synthetic fragment 2–15 in water and in trifluoroethanol are shown in the fig. 1. The spectrum in water (pH 5.75) is characterized by a large negative band at about 198 nm, by a small positive band at 217 nm and by an additional negative band centered around 228 nm. The general features are those typical of largely disordered polypeptides. In the presence of trifluoroethanol the negative band originally present at 228 nm in water is shifted to 222 nm, the typical wave-

* When peptides containing N^G-nitroarginine or *S*-*p*-methoxybenzylcysteine were hydrolyzed the value of arginine was taken from the sum of arginine and ornithine and cysteine was determined as cysteic acid after performic acid oxidation.

* The *S*-acetamidomethylcysteine is decomposed during the hydrolysis and gives several peaks emerging from the 50 cm column of the amino acid analyzer (Carlo Erba mod. 3A-27) at the same volume of aspartic acid, serine, threonine and cysteine.

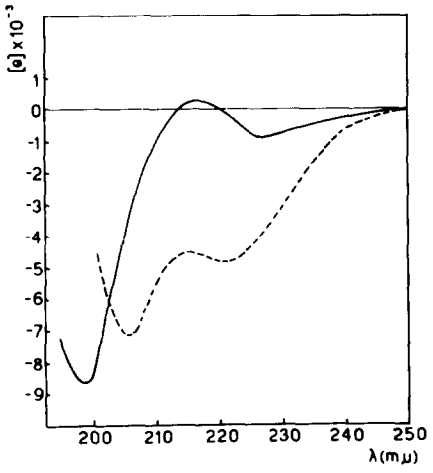

Fig. 1. Circular dichroism spectra of the synthetic 2–15 *N*-terminal fragment of hen egg-white lysozyme in water (——) and 50% trifluoroethanol (- - - -).

lenght of the amide n-π^* transition of the α-helix. Furthermore the small positive band is abolished and the large negative band, centered at about 198 nm in water, associated with the amide π-π^* transition of disordered polypeptides, is replaced by another negative band which, in 50% trifluoroethanol, appears at 207–208 nm, the typical wavelenght of the amide parallel transition of the α-helix. It is evident that the 2–15 sequence of lysozyme is not helical in water but is able to undergo a solvent-induced conformational transition. This behaviour is strictly paralleled by ribonuclease S-peptide [2–4].

The results obtained by studying isolated and/or synthetic *N*-terminal fragments of ribonuclease and of lysozyme suggest that long-range interactions are needed in a protein molecule as stabilizing points for the native conformation. The polypeptide chain is thus able to assume its ultimate conformation only when the synthesis of that part of the molecule which allows the existence of these interactions is complete.

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