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A PARTIALLY SYNTHETIC HIGHLY ACTIVE RIBONUCLEASE S ANALOGUE IN WHICH L-HOMOHISTIDINE REPLACES HISTIDINE-12 AS THE OPERATIVE COMPONENT[1,2]

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The discovery of Richards[3] that the two parts into which ribonuclease A can be split, the S-protein and the relatively short S-peptide, combine to yield the fully active ribonuclease S', opened a way to systematic variation of the molecular structure of an enzyme with nowadays potential of polypeptide synthesis During the past decade important advances on this basis have been made in the exploration of the possible role of each of the first 20 amino acids of ribonuclease in the enzymatic process[4,5]. One may expect that a continued effort along this line, combined with the results obtained by systematic variation of the substrate and the information deduced from physical measurements, X-ray diffraction studies in particular, will lead to an understanding of the precise mechanism of the steps of an enzymatic reaction that equals or even surpasses our insight into the mechanism of reactions of small and medium-sized organic molecules

There are good indications that the imidazolyl groups of His-12 and His-119 function as the proton abstractor/donators that catalyse the splitting of P-O bonds of RNA or comparable substrates[6,7] Replacement of His-12 by the β-(pyrazolyl-3)-L-alanyl[8] or 4-fluoro-L-histidyl residue[9] leaves the capacity to bind to S-protein unimpaired but results in an inactive ribonuclease S analogue These findings strongly support the hypothesis on the role of His-12, the two substitutes replace histidine fittingly from a steric point of view, however they have a considerably lower pK_a and cannot function as an efficient proton donator/acceptor at neutral pH.

Having worked out a convenient synthesis of L-homohistidine[10,11], an amino acid complementary to the two substitutes mentioned in that it is very similar to histidine with regard to its pK_a but different in steric aspects, we extended our study of synthetic S-peptide analogues[1] by incorporating Hhi at position 12.

(Hhi[12])-RNase S-peptide 1-14 was synthesized using the solid-phase method of Merrifield[12,13] starting from 1,5 g (0,48 mmol) Boc-Asp (OBzl)-resin (copolystyrene-1%-divinylbenzene). α-Amino groups were protected with the Boc group. The following side chain blocked derivatives were

used Hhi (DNP), Arg(NO_2), Glu(OBzl), Asp(Obzl), Lys(Z), Thr(Bzl). Completeness of each coupling step was controlled by means of the ninhydrin test of Kaiser[14]. After removal of the N^{im}-DNP group with thiophenol[15] the protected tetradecapeptide resin was treated with liquid HF[16] and the crude peptide was isolated by extraction with 1N acetic acid followed by lyophilization Purification of the crude product was effected by preparative free-flow electrophoresis followed by cation-exchange chromatography on SP-Sephadex C-25 using an ammonium acetate gradient (0,05M to 0,15M, pH 5,0). The fractions containing the main peak were pooled and lyophilized yielding a mixture of the tetradecapeptide and its oxidation product (containing methionine sulfoxide). Reduction of this mixture with thioglycolic acid[17] gave 94 mg of a chromatographically homogeneous product. Amino acid analysis Lys 2 09, Glu 3 37, Thr 1 00, Ala 3 00, Phe 0.93, Arg 0 91, Hhi 0 98, Met 0 91, Asp 1.03. A sample of the natural sequence 1-14 was synthesized along the same route in order to serve as a comparison.

The capacity of the synthesized tetradecapeptides to generate catalytic activitythrough combination with RNase S-protein was measured as described by Berger and Levit[18] (substrate yeast RNA). The (Hhi^{12})-RNase S-peptide 1-14 shows a capacity to combine with S-Protein that is significantly less than that of the natural sequence (ratio of K_b values about 1 200) However the (Hhi^{12})-RNase S' analogue formed has practically full enzymatic activity, compared to the complex from S-protein and the natural sequence 1-14 (activities are ∿ 70% and ∿ 80% respectively of that of RNase S'). To our knowledge this is the first instance where a modification of the His-12 residue does not entail decreased enzymatic activity. Significantly here, in contrast to the substitutions by fluorohistidine and pyrazolyl alanine, histidine is replaced by an amino acid with similar acid/base properties of the side chain.

It is clarifying to correlate these results with those obtained in the study of the poly-peptide hormone Angiotensin II. The replacement of histidine by homohistidine gave a drastic decrease in myotropic activity[19], whereas replacement of histidine by pyrazolylalanine is reported to result in a moderate decrease only[20]. It was tentatively concluded that in the action of Angiotensin II the histidine residue does not take part in a chemical conversion but rather functions in the binding to the receptor[19].

Interestingly there seems to be sufficient flexibility in and around the active site of ribonuclease to allow that, with homohistidine in the place of histidine, still the optimal geometric arrangement for the enzymatic reaction can be realized. This may also explain the activities reported for the N-carboxymethylhistidine-12-ribonucleases[8,21].

A more detailed discussion of the consequences for the mechanistic picture will be offered in connection with results on further modifications at the active site of ribonuclease now under investigation[22]. An X-ray analysis of the complex of a quasi substrate and (Hhi[12])-RNase S' should be highly illuminating.

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