Structural similarities among concanavalin A, haptoglobin, and trypsin

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Received 3 April 1984

Comparison of the primary structure of concanavalin A with those of trypsin and β (heavy) chain of haptoglobin revealed a significant degree of chemical similarity. The amino acid sequence of concanavalin A was found to be 53.6% related to trypsin and haptoglobin. Certain analogy in the tertiary structure was shown by the use of thionine binding to the specific active site, characteristic for serine proteases.

Concanavalin A *Haptoglobin* *Trypsin* *Structural similarity*

1. INTRODUCTION

In a previous paper [1] Con A, a plant lectin, was found to contain 25 tripeptide sequences of amino acids identical as present in human Hp, a genetically determined serum α_2-glycoprotein. Hp of genetic type 1-1 is a tetramer, composed of two α (light) chains and two β (heavy) chains, linked by disulphide bridges [2]. Amino acid sequence analysis of the Hp β chain pointed to its chemical similarity to the chymotrypsinogen family of serine proteases [3]. This homology was also confirmed in the three-dimensional structures of trypsin and Hp [4,5].

The aim of this paper was to determine whether any homology exists between Con A and the family of serine proteases as represented by trypsin and Hp.

2. MATERIALS AND METHODS

Human Hp type 1-1 was prepared from ascitic fluids [6]. The preparations used for the experiments were over 95% pure as checked by polyacrylamide gel electrophoresis and spectrophotometry. Con A from *Conavalia ensiformis,* BAEE, TLCK, and TPCK were products of Serva (Heidelberg). Bovine trypsin was from Carl Roth (Karlsruhe), and thionine (3,6-diaminophenothiazine) from BDH (Poole, England). Other reagents were purchased from P.O.Ch. Gliwice (Poland).

Thionine binding was performed as in [5]. A solution of thionine (3×10^{-5} M) was buffered with 0.05 M potassium phosphate at pH 7.1 and 0.05 M glycine–KOH at pH 9.5 (for trypsin). Measurements were made on a Specord UV VIS spectrophotometer at 580–870 nm. The sample cell contained thionine and a 6×10^{-4} M solution of Hp, trypsin or Con A. The reference cell contained thionine alone. In the experiments with BAEE and inhibitors, solutions of thionine (6×10^{-5} M) were placed in both the sample and reference cells, while Con A (6×10^{-4} M) was added only to the sample cell. BAEE or inhibitor (6×10^{-3} M) was added to both the sample and reference cells.

Abbreviations: Con A, concanavalin A; Hp, haptoglobin; Hp β, haptoglobin β (heavy) chain; BAEE, benzoyl arginine ethyl ester; TLCK, L-1-chloro-3-*p*-tosylamido-7-amino-2-heptanone; TPCK, L-1-chloro-3-tosylamido-4-phenyl-2-butanone

3. RESULTS

Alignment of amino acid sequences of Con A (237 amino acids) to Hp β (245 amino acids) and to trypsin (223 amino acids) shows significant chemi-

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00145793/84/$3.00

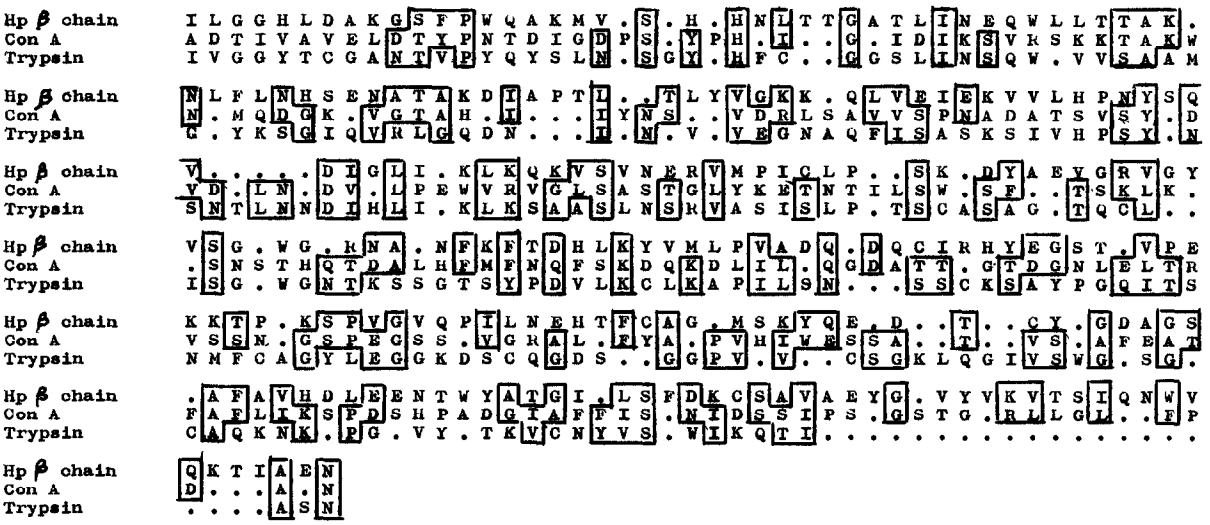

Fig.1. Comparison of amino acid sequences of concanavalin A (Con A), to the β chain of human haptoglobin (Hp) and bovine trypsin. A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. Chemically similar residues: I = L = V; G = A; T = S; Y = F = W; D = E = N = Q; K = R. Chemically similar and identical residues are boxed. Amino acid sequences of Hpβ chain and trypsin were taken from [3], and Con A from [7].

cal similarity in both cases (fig.1). Con A was found to contain 84 amino acids common with Hpβ, and 83 common with trypsin, 40 of them being present simultaneously in respective positions in the 3 proteins. This means that Con A contains 127 amino acids (53.6%) related to either Hp or trypsin.

The 3 proteins also show a certain similarity in the content of amino acids as grouped according to polarity (table 1). In the cases of polar and weak amino acids, differences between Con A and trypsin are lower than between Hpβ and trypsin, in spite of the latter well-known homology [3].

These results suggested a verification of eventual functional properties of Con A, that could have been connected with the observed relationships in the primary structures. The presence in Hp of a specific dye-binding site, characteristic for serine

Table 1

Amino acids of haptoglobin β chain, concanavalin A (Con A), and trypsin as classified according to their polarity

Groups of amino acids		Polarity	Percentage content in		
			Hpβ	Con A	trypsin
Polar	Arg, Lys, His, Gln	10.4–13.0	33.5	28.7	24.7
	Asn, Asp, Glu		(82)	(68)	(55)
Weak polar	Ala, Pro, Gly	8.0–9.2	31.0	40.5	40.8
	Thr, Ser		(76)	(96)	(91)
Non-polar	Cys, Val, Met, Ile	4.9–6.2	35.5	30.8	34.5
	Leu, Phe, Tyr, Trp		(87)	(73)	(77)

The grouping and polarity of amino acids were taken from [8]. Number of amino acids is given in parentheses

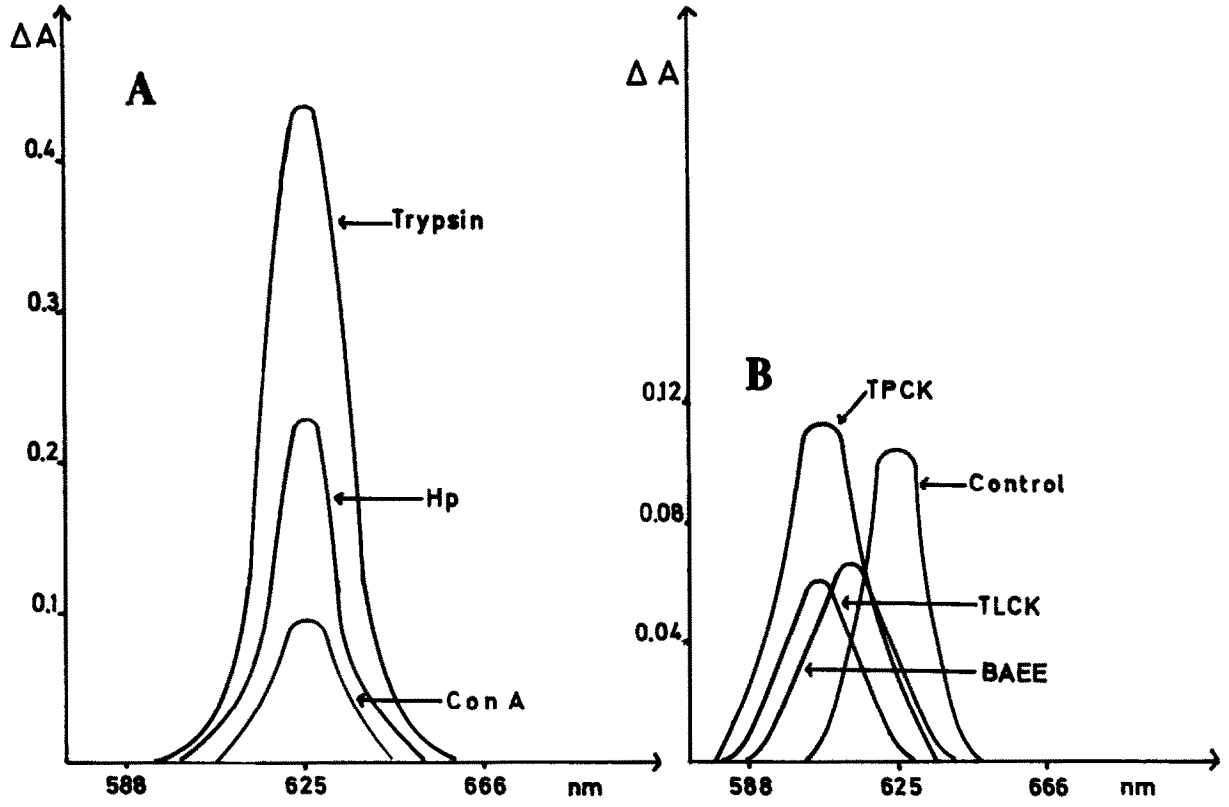

Fig.2. The difference spectra of (A) thionine with Con A, Hp, trypsin, and of (B) Con A–thionine complex with BAEE, TLCK, TPCK. For details see section 2.

proteases, was recently reported [5]. It prompted us to look for such a site in Con A. In fig.2 the difference spectrum for the interaction of Con A with thionine as compared with the respective spectra of trypsin and Hp shows a resemblance in shape, the maximum occurring at 625 nm. The amplitude of the maximum rose from Con A through Hp to trypsin. Competition experiments as carried out with BAEE, TLCK and TPCK (fig.2B) resulted in a blue-shifted maximum at 612, 602 and 604 nm, respectively. If ΔA of the maximum for the control sample is assumed to be 100%, that of the TPCK sample would be 100.8%, BAEE 63.4%, and TLCK 57.7%. This means that BAEE, a trypsin specific inhibitor, did compete with thionine binding by Con A, whereas TPCK, a chymotrypsin-specific inhibitor, had practically no effect on the reaction.

4. DISCUSSION

Numerous protein families based on more or less distinct homologies have been reported, inter alia: albumin and α-fetoprotein [9]; tonin, nerve growth factor α-subunit, epidermal growth factor-binding protein and serine proteases [10]; streptokinase and serine proteases [11]; 'superfamily' of ovalbumin, antithrombin III and α_1-proteinase inhibitor [12]. During identification of β-turns in regions of polypeptide chain reversals in Hp, some analogies with Con A emerged [1]. The positions corresponding to the proteolytic active site residues of trypsin, His-57 and Ser-195 (replaced in Hpβ by Lys and Ala, respectively) [3], exist in Con A as His-51 and Ser-189. The trypsin-specific residue Asp-189 as well as Asp-102 and Asp-194 (forming an internal ion pair with the α-amino terminal resi-

due in serine proteases), present also in Hp, have their equivalents in Con A as Glu-183, Glu-102 and Glu-192, respectively. Moreover, the hydrophobic cavity formed in Con A by Leu-81, Val-89, Phe-111 and Ser-113 [13] was found in Hp to be Leu-88, Val-95, Val-117 and Val-120, respectively. It seemed extremely unlikely that such similarities occurred purely by chance. Here, evident relationships in the primary structures of Con A, Hp and trypsin have been demonstrated, the anticipated resemblance in the three-dimensional structures being proved in the experiment with difference spectra of thionine – Con A.

Examples of homologous proteins shared by plants and other organisms are not too rare and incude serine proteases. Con A as related to Hp and trypsin seems to represent a striking example of a homologous protein with a phylogenetically distant origin and apparent lack of functional similarity. However, Hp was found to inhibit Con A-induced lymphocyte transformation [14]. The molecular basis of this phenomenon as well as other biological effects that might result from the chemical similarity of Hp and Con A are being studied in our laboratory. From an evolutionary viewpoint, the question arises as to whether the carbohydrate-binding properties of Con A, analogously to that of haemoglobin binding of Hp, were acquired after divergence from a serine protease common ancestral gene.

It is accepted that during evolution the number of truly independent, new protein families is very small, most 'novelties' in protein evolution being variations of available proteins [15]. Therefore, the observed homology suggests the incorporation of Con A in a new superfamily, demonstrating distant divergences preceding most vertebrate evolution.

ACKNOWLEDGEMENT

This work was supported by grant II.1.2.3 from the Polish Academy of Sciences.

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