THE COLLAGENOUS PART OF C1q IS UNAFFECTED IN THE HYDROXYLYSINE-DEFICIENT COLLAGEN DISEASE

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1. Introduction

A well documented genetic deficiency of an enzyme post-translationally acting on specific peptidyl amino acids occurs in Ehlers-Danlos syndrome type VI, clinically characterized by ocular fragility, severe progressive scoliosis, hyperextensible skin, marked joint laxity with recurrent dislocations, and easy bruising [1]. In skin, the biochemical marker of this autosomal recessively inherited syndrome is a reduction of the Hyl level with an unaffected Hyp concentration [2–7]. In fibroblast cultures, only the Hyl content but not the Hyp content of collagen (types I,III) is lowered [6,7], the activity of the Hyl synthesizing enzyme being ~10% of normal. The activity of the Hyp producing prolylhydroxylase (EC 1.14.11.2) is unaffected [2–9]. As hydroxylysyl-derived crosslinks of collagen are more stable than those derived from lysyl residues [10], and as the number and chemical nature of the crosslinkages in collagen from Ehlers-Danlos syndrome type VI patients differ totally from the normal pattern with a concomitant increase in solubility [11], the diminished activity of lysylhydroxylase (EC 1.14.11.4.) has been proposed as the molecular basis of this inherited connective tissue disease [12].

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Abbreviations: Hyp, 4-transhydroxyproline; Hyl, erythro-5-hydroxylysine; EDTA, ethylene diaminetetraacetic acid; VBS-S, veronal buffered saline–sucrose

C1q contains a collagen-like sequence of ~80 amino acid residues which imparts to this serum protein some of the biophysical and biochemical characteristics of collagen [13–16]. Both C1r and C1s interact with C1q through its collagen microfibril-like part, constituting the first complement component C1; selective destruction of the collagenous triple helical structure within C1q obviates this association [17–19]. Following the binding of immune complexes to the non-collagenous heads of C1q, a conformational change is thought to occur in its collagenous tail resulting in the activation of C1r–C1s and consequently of the entire complement cascade [13].

We undertook this study of the classical pathway of complement in a family affected by Ehlers-Danlos syndrome type VI to determine whether, in addition to the documented abnormalities in interstitial collagens, the structure or function of the collagenous part of C1q is impaired.

2. Materials and methods

2.1. *Amino acid analysis of euglobulins*

Venous blood was allowed to clot at 4°C. After centrifugation, serum was stored at −25°C. Serum was diluted 1:8 with deionized water at 0°C and adjusted to pH 5.6 with 1 N HCl under constant stirring. After 12 h, the precipitate was collected (15 000 rev./min, 45 min, 4°C), washed in 0.02 M acetate buffer (pH 5.6) and defatted in acetone. The final precipitate was dried. Samples were hydrolysed in 2 ml constant boiling 6 N HCl at 110°C for 22 h under nitrogen in sealed tubes. The acid was removed

under vacuum and the samples used for amino acid analysis on a Beckman Multichrom C analyser. Hyp was developed from a 0.9 X 52 cm column (Beckman M 72 resin) with a 0.2 N Na-citrate buffer (pH 3.15) at 30°C, while Hyl was eluted from a 0.9 X 12 cm column with 0.2 N Na-citrate, 1 N NaCl (pH 6.28) at 55°C.

2.2. *Hemolytic assay of complement components*

The measurement of whole serum hemolytic complement (CH50) was performed by the standard procedure [20]. The functional activity of the individual components C1,C4 and C2 was determined using erythrocytes in the state EAC4, EAC1 and EAC14. The details for the preparation of these intermediates, the appropriate buffers and the titration proper are given in [21]. The functional assay for C1q was performed on serum samples diluted 1:1 in 0.05 M EDTA. 0.1 ml of this mixture was incubated with 0.1 ml EA (1.3×10^8 cells/ml) for 15 min at 30°C. Then the cells were washed twice and resuspended in 0.2 ml VBS-S in the presence of 0.1 ml recalcified RC1q for 15 min at 30°C. This reagent, source for C1r and C1r, was prepared according to [22]. The cells were washed again, sensitized with C4 and subsequently treated as detailed for the C1 test. Guinea pig C1 was functionally purified as in [23]. C4 and C2 were purchased from Cordis Co., Miami, FL. Guinea pig serum served as source for C-EDTA.

3. Results

3.1. *Analytical data*

All the C1q in serum precipitates with the euglobulin fraction in which it is the only protein containing post-translationally hydroxylated amino acid residues [24]. For determination of the Hyl/Hyp ratio it is therefore sufficient to analyse the euglobulins. Moreover, this coefficient is independent of varying C1q concentrations in the different euglobulin preparations. In collagen biochemistry, this parameter has been used for the differentiation of interstitial collagen types [25] and for the study of the Hyl content in tissues from Ehlers-Danlos syndrome type VI patients [2]. As expected (table 1), the Hyl/Hyp ratio found in the control euglobulin fraction equals the values for purified human C1q calculated from the data of different authors. A significant diminution of the coefficient in the euglobulins of the probands could not be established. The values for the homozygous patient, the heterozygous mother and the heterozygous father were close to the control and to the ratio reported for purified human C1q. Thus, in this Ehlers-Danlos type VI family the structural integrity of the collagen-like part within C1q appears to be unaffected. The hydroxylation of lysyl residues in this serum protein is unaltered, irrespective of the homo- or heterozygous state for lysylhydroxylase deficiency in fibroblasts.

Table 1
Hyl/Hyp ratios

	C1q	Skin collagen
Literature	0.46[a]	0.06[c]
	0.53[b]	
Control	0.52	0.053[d]
Patient	0.48	No Hyl[d]
Mother	0.61	0.022[d]
Father	0.44	0.022[d]

[a] Purified according to [30]
[b] Purified according to [31]
[c] [25]
[d] Calculated from the data in [7]

Table 2
Activity of whole complement and of individual complement components

Parameter	Control	Patient	Mother	Father
CH50 (U/ml)	30.3	29.3	29.5	28.3
(Eff. mol./ml)				
C1q ($\times 10^{13}$)	0.21	0.19	0.23	0.18
C1 ($\times 10^{13}$)	3.1	3.0	3.4	3.2
C4 ($\times 10^{13}$)	1.7	1.4	1.6	1.9
C2 ($\times 10^{10}$)	2.8	1.6	1.4	1.4

3.2. *Functional data*

The whole serum hemolytic complement, measured in terms of CH50 units, and the hemolytic assays of the individual classical pathway components C1q,C1 and C4 did not show a reduction. Table 2 demonstrates that C1q and C1 are unaffected in their activities. C2 in these three sera, however, reached ⩽60% of the control value. The data for C1q indicates that the ability to utilize C1r and C1s, offered externally in the form of RC1q, is unaffected.

4. Discussion

The family whose C1q we investigated here was the subject of a connective tissue study [7] biochemically characterizing the dermal collagen of the 3 year old boy and his parents. These findings led to the diagnosis of Ehlers-Danlos syndrome type VI in the boy as his skin collagen revealed a complete lack of hydroxylysine, while that of his parents contained only 50% the amount found in healthy individuals. The Hyl/Hyp ratios calculated from these data are also given in table 1.

In this autosomal recessively inherited, hydroxylysine-deficient collagen disease an analytical and functional study of C1q suggested itself for several reasons.

(1) Fibroblasts produce and secrete C1q [26], and these cells synthesizing most of the interstitial collagen were supposed to be a major source of the C1 hemolytic activity in serum [27].
(2) In contrast to dermal collagen, for which a half-life of ~60 days was found [28], the C1q turnover is rapid with a daily plasma pool fractional catabolism of 65% [29].
(3) As the hydroxylysine content of the collagen-like part within C1q is exceptionally high [30–32], the Hyl/Hyp index represents a sensitive indicator for the level of hydroxylysine in C1q.

Hence, a diminution of the lysylhydroxylase activity in fibroblasts, marker for Ehlers-Danlos syndrome type VI, should result in a clear cut effect on the parameters measured here.

Surprisingly, we did not find any compositional or functional alteration of C1q, in the homocygotic patient or in the heterocygotic parent serum (see tables 1,2).

Three explanations seem worth considering:

(1) A mutation of the structural gene affects the activity of lysylhydroxylase towards collagen, but not towards C1q. This might be due to an alteration in its substrate binding site, or subsites, or due to a change in the requirement for cofactors, e.g., ascorbate. A lowered affinity of the mutant enzyme for vitamin C has been reported [33], and a concomitant reduction in enzyme activity in vivo could, for structural reasons, preferentially be reflected in collagen.
(2) A mutation of the regulator gene reduces the number of the catalytically active lysylhydroxylase molecules, upsetting the balance with prolylhydroxylase. Consequently, collagen becomes triple helical before the hydroxylation of lysyl residues is completed. As the triple helical conformation prevents further hydroxylation of lysyl residues [39], the Hyl/Hyp ratio will remain low. The rate of triple helix formation of cartilage type II collagen is slower than that of skin type I, leaving more time for Hyl synthesis [34]. In fact, in a patient suffering from Ehlers-Danlos syndrome type VI the Hyl/Hyp ratio of cartilage was normal while that of skin was significantly lowered [2]. A slow rate of triple helix formation might occur in C1q synthesis, too. Interestingly, the collagen-like regions of C1q are bent halfway along their length as in one of the peptide chains a glycine residue in the typically collagenous sequence –X–Y–Gly– is replaced by alanine [35], producing a distortion of the triple helix and possibly a delay in its formation.
(3) An isoenzyme of lysylhydroxylase may exist specific for C1q synthesizing cells that is unaffected by the genetic defect occuring in collagen synthesizing cells. Such a dissociation of C1q biosynthesis from collagen biosynthesis also is suggested by the C1q insensitivity to acute vitamin C deficiency in guinea pigs [36]. C1q and C1 structurally and functionally remained unaffected, while the interstitial collagen synthesis was markedly impaired due to the reduction in the ascorbate-dependent prolylhydroxylase activity. Nevertheless, direct inhibition of prolylhydroxylase in C1q synthesizing cells from guinea pigs stops C1q secretion [37] and demonstrates a conformation-dependent barrier in these cells [38] that is well-known from inhibition studies of prolylhydroxylase in collagen synthesizing cells: the secretion of Hyp-deficient protein is reversibly stopped at 37°C, but resumed at 20°C

where the triple helical conformation is stable independent of the Hyp content.

We interpret the data on C1q presented here and in [36–38] as indicating a non-identity of the post-translational hydroxylase activities involved in the biosynthesis of C1q and of collagen.

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