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Structural and biological characteristics of connective tissue activating peptide (CTAP-III), a major human platelet-derived growth factor*

(glycosaminoglycan/mitogenesis/β-thromboglobulin/low-affinity platelet factor 4)

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ABSTRACT **Connective tissue activating peptides (CTAPs) extracted from leukocytes and platelets stimulate glycolysis and synthesis of glycosaminoglycan and DNA in cultured human connective tissue cells. CTAP-III, isolated from fresh or outdated human platelets, is a low molecular weight single-chain protein with an isoelectric point of 8.5 that markedly stimulates DNA synthesis and multiple aspects of glycosaminoglycan and proteoglycan metabolism. This report presents a definitive comparison of CTAP-III prepared by two methods [one designated (A), alternative] with similar platelet proteins described by others, β-thromboglobulin (β-TG) and low-affinity platelet factor 4 (LA-PF-4). CTAP-III, CTAP-III(A), LA-PF-4, and β-TG have common antigenic determinants documented by immunoprecipitation and radioimmunoassay. CTAP-III, CTAP-III(A), and LA-PF-4 are biologically active in that they stimulate DNA and glycosaminoglycan synthesis by human synovial cells; β-TG is inactive. Carboxyl-terminal digestion gave identical terminal sequences for CTAP-III, CTAP-III(A), and β-TG. Amino-terminal sequence data indicate that CTAP-III and CTAP-III(A) (also LA-PF-4) are identical and differ from β-TG only by an additional amino-terminal tetrapeptide (Asn-Leu-Ala-Lys-). The biologically active molecule, CTAP-III, may be proteolytically converted to its inactive degradation product (β-TG) in the course of platelet aging, platelet storage, release from the platelets, or initiation of biological activity.**

Nearly a century of experience indicates that growth of animal cells in culture is facilitated by biologically active components found in serum harvested from clotted whole blood. Growth of cells in culture is not, however, supported by "plasma-serum" produced by recalcification of cell-free plasma (1). On the other hand, "plasma-serum" produced by the recalcification of platelet-rich plasma functions in a manner similar to serum (2). It is clear that macromolecules derived from platelets may be viewed as the principal mitogenic factors of serum, and several different purified preparations have been reported (3–10).

The platelet proteins whose sequences have been determined previously have no known growth-promoting activity; these include platelet factor 4 (PF-4) and β-thromboglobulin (β-TG). Both are stored in platelet α granules and secreted upon platelet aggregation (11). The complete covalent structure of human β-TG shows an 81-residue protein with an isoelectric point of 7.0, a low affinity for heparin (12), and no known biological activity. PF-4 is a 70-residue protein (13) with a high affinity for heparin (9) and approximately 50% primary structure homology with β-TG.

Information concerning biologically active platelet proteins attended purification procedures guided by bioassays, resulting in the detection of more than one biologically active platelet factor. In addition to mitogenic properties, some biologically active platelet factors were found to stimulate extracellular matrix (glycosaminoglycan) synthesis (3–6, 14). "Connective tissue activation" was originally defined to include stimulation of glycosaminoglycan (GAG) synthesis and glycolysis (15), a concept subsequently broadened to include mitogenesis when factors derived from polymorphonuclear leukocytes and platelets were shown to stimulate DNA synthesis as well as GAG synthesis (3, 5, 16). Proteins capable of activating connective tissue cells have been collectively termed "connective tissue activating peptides" (CTAPs), and the terminology was tentatively coded to indicate the cell or tissue of origin. Thus, CTAP-I is of lymphoid origin (6), CTAP-II of tumor cell origin (6), CTAP-III (5, 6) and CTAP-P_2 (14) of human platelet origin, and CTAP-PMN (16) is from polymorphonuclear leukocytes.

CTAP-III isolated from outdated human platelets was shown to share amino acid compositional, electrophoretic, and antigenic properties with β-TG (6). The biological activities demonstrated for CTAP-III included stimulation of DNA, hyaluronic acid, and sulfated GAG synthesis, stimulation of glycolysis, prostaglandin E_2 secretion, and intracellular cAMP accumulation (5, 6, 17–19).

This report defines the primary structure of CTAP-III, a major platelet-derived growth factor. A part of these data has appeared in preliminary form (8). We also present an alternative method for isolating the same biologically active protein [referred to as CTAP-III(A)] from outdated human platelets. CTAP-III(A) is a protein with biological and chemical characteristics identical to those of CTAP-III, thus demonstrating another isolation scheme leading to the same defined platelet-derived growth factor.

MATERIALS AND METHODS

Platelet Proteins. CTAP-III was prepared as previously reported (6) and summarized below. In *method 1*, active protein from outdated blood bank platelets was extracted into acid/ethanol and precipitated with acetone. Dialysis against 1.0 M acetic acid containing 3.6% sodium chloride precipitated additional inactive protein. The supernatant fluid from this step was chromatographed over a Sephacryl S-300 column (5 × 55 cm; Pharmacia) equilibrated with neutral phosphate-buffered

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Abbreviations: CTAP-III, connective tissue activating peptide III; CTAP-III(A), CTAP-III prepared by alternative methods; β-TG, β-thromboglobulin; PF-4, platelet factor 4; LA-PF-4, low-affinity PF-4; GAG, glycosaminoglycan.

* This is paper no. 24 in the "Connective tissue activation" series. The previous paper is ref. 37.

saline, and the material emerging at the elution position of cytochrome *c* was retained and applied to a CM-Sephadex C-50 column (2 × 5 × 8 cm; Pharmacia) equilibrated with 0.1 M phosphate buffer, pH 5.0, containing 0.05 M sodium chloride. Biologically active fractions were eluted when the pH of this buffer was increased to 6.25–6.85; these were pooled and reapplied to a CM-Sephadex C-50 column (2.5 × 8 cm) equilibrated with 0.1 M sodium phosphate buffer, pH 6.1. Batchwise elution with sodium chloride at increasing concentrations displaced CTAP-III at 0.25 M sodium chloride. Such preparations were dialyzed against 0.1 M NH_4HCO_3 and lyophilized.

Alternatively, in *method 2*, CTAP-III(A) preparations were from outdated platelets heated to 75°C for 10 min and centrifuged at 6,000 × *g* for 30 min. The supernatant fluid was filtered, mixed with an equal volume of 0.1 M sodium phosphate/0.15 M NaCl, pH 6.0, and applied to a 2.5 × 25 cm column containing equilibrated CM-Sephadex C-50. The column was washed with diluent buffer, with phosphate buffer containing 0.15 M NaCl, and finally with phosphate buffer containing 0.5 M NaCl. Column effluents were monitored for protein (280/206-nm absorbance) and aliquots were radioimmunoassayed for β-TG and PF-4 (20). The 0.5 M NaCl fractions, which contained β-TG but not PF-4, were pooled, dialyzed, and freeze-dried. This preparation was subsequently dissolved in 0.1 M NH_4HCO_3 and filtered through a Sephadex G-75 column (2.5 × 92 cm; Pharmacia).

β-TG was kindly provided by Duncan S. Pepper (Regional Centre, Royal Infirmary, Edinburgh, Scotland) (21). All three proteins [CTAP-III, CTAP-III(A), and β-TG] were evaluated for homogeneity by sodium dodecyl sulfate gel electrophoresis (22) and acid/urea gel electrophoresis (23).

Structural Studies. *Peptide maps.* Peptide maps of CTAP-III and β-TG were prepared as follows: three hundred micrograms of CTAP-III or β-TG was digested by trypsin for 14 hr in 0.2 M NH_4HCO_3, pH 7.1, and freeze-dried. The sample was dissolved in 0.05 M pyridine acetate, pH 5.0, and applied to a 20 × 20 cm cellulose sheet at a marked position. After 1.5 hr of electrophoresis and drying at room temperature, the sheet was rotated 90° and chromatography was carried out in 1-butanol/pyridine/acetic acid/water, 15:10:3:1 (vol/vol), for 8 hr. The plate was dried and sprayed with ninhydrin, and the peptides were circled.

Amino acid sequence analysis. Amino acid sequences were determined by automated phenyl isothiocyanate degradation with a Beckman 890C sequencer and a modified *N,N*-dimethylallylamine protein program as described (24). Phenylthiohydantoin derivatives of amino acids were qualitatively identified by using TLC (25) and quantitated by using a HPLC system. Phenylthiohydantoin derivatives from the sequencer analyses were dissolved in absolute methanol and injected onto a Partsil PXS octadecylsilyl column (Whatman). An isocratic elution was achieved by using a Micromeritics (Norcross, GA) model 7000 chromatograph to blend weak (0.1 M sodium acetate, pH 4.5) and strong (methanol) solvents at a volume ratio of 59:41 (weak/strong). Derivatives were detected by absorbance at 254 nm, using a Micromeritics 785 Chromonitor. Elution positions were compared to those of phenylthiohydantoin standards (Pierce) and quantitated by measuring the peak area relative to norleucine phenylthiohydantoin standard injected with each unknown.

Prior to automated sequence analysis, the proteins were reduced with dithiothreitol and carboxymethylated (13) with iodo[^{3}H]acetic acid (New England Nuclear, NET 147, 100 mCi/mmol; 1 Ci = 3.7 × 10^{10} becquerels). Half-cystine residues were identified by liquid scintillation counting of 5% of each phenylthiohydantoin derivative in a Packard counter. Solvents

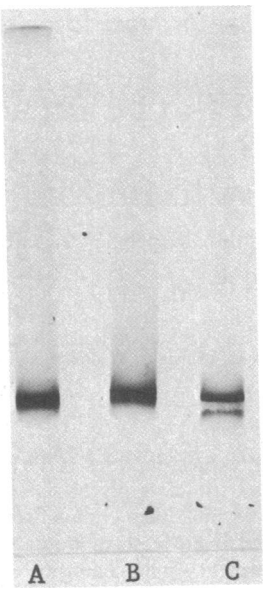

FIG. 1. CTAP-III (lane A), CTAP-III(A) (lane B), and β-TG (lane C) (25 μg each) were compared electrophoretically in a polyacrylamide acid/urea gel system. The preparations appeared essentially homogeneous and possessed similar mobilities in this system.

used for the sequencer were purchased from either Beckman or Pierce and used without additional purification. Solvents for HPLC were purchased from Burdick and Jackson (Muskegon, MI).

Carboxyl-terminal amino acids were determined by using carboxypeptidase Y (26). Aliquots (50 nmol) of platelet protein were removed at designated time intervals, the protein was removed by trichloroacetic acid precipitation, and the free amino acids were identified by analysis on a Beckman model 121 M analyzer.

GAG Synthesis. Synthesis was measured by utilizing microcultures of human synovial cells to assay biologically active substances by assessing their capacity to stimulate incorporation of [^{14}C]glucosamine, [^{14}C]glucose, or $^{35}SO_4^{2-}$ into hyaluronic acid and sulfated GAGs (27). Isotope incorporation is used as a measure of GAG synthesis; lability to specific carbohydrases (*Streptomyces* hyaluronidase, chondroitinases AC and ABC) permits qualitative identification. Protein was measured by a colorimetric method (28).

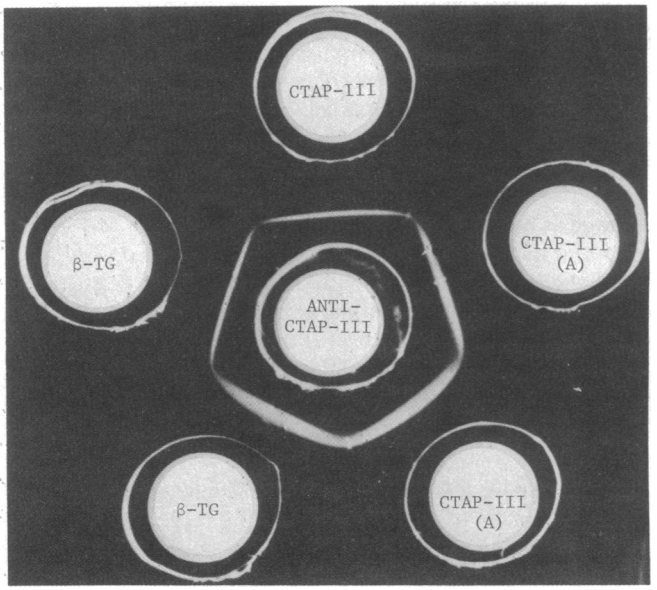

FIG. 2. Anti-CTAP-III antiserum reacts in double immunodiffusion to give fused precipitin bands linking CTAP-III, CTAP-III(A), and β-TG.

Table 1. Comparison of CTAP-III, CTAP-III(A), and β-TG with respect to stimulation of GAG and DNA synthesis

Preparation*	Protein, μg/ml	[^{14}C]GAG synthesis, cpm/100 μl medium from 2 × 10^4 cells			[^{3}H]DNA synthesis, cpm/10^4 cells		
		Control	Experimental	Experimental/ control	Control	Experimental	Experimental/ control
CTAP-III (1)	25	920 ± 160	2,400 ± 600	2.56	1,700 ± 300	6,200 ± 600	3.64
CTAP-III (2)	70	920 ± 160	10,500 ± 2,100	11.38	1,700 ± 300	10,000 ± 800	5.92
CTAP-III(A) (1)	91	920 ± 160	2,100 ± 500	2.29	1,700 ± 300	6,500 ± 1,100	3.82
CTAP-III(A) (2)	73	920 ± 160	6,700 ± 300	7.23	1,700 ± 300	36,600 ± 4,600	21.60
β-TG	252	920 ± 160	1,000 ± 100	1.07	1,700 ± 300	1,400 ± 200	0.85

Data are expressed as mean ± 1 SD for four microculture wells in each group.
* Numbers in parentheses indicate different platelet preparations.

Mitogenic Activity. Activity of test substances was measured by adding tritiated thymidine (51.7 Ci/mmol) to human synovial cultures and determining the radioactivity in cell lysates (5, 6).

RESULTS

Electrophoretic, Immunologic, and Bioassay Studies. CTAP-III, CTAP-III(A), and β-TG appeared homogeneous on sodium dodecyl sulfate/polyacrylamide gels, each exhibiting a molecular weight of approximately 10,000; acid/urea gels (Fig. 1) also indicated that a single protein was present in each preparation. Immunodiffusion studies show that the antigenic determinants of CTAP-III are shared by CTAP-III(A) and β-TG (Fig. 2). Earlier immunodiffusion asssays showed that antigenic determinants of CTAP-III and β-TG were also shared with LA-PF-4 (6). Furthermore, a radioimmunoassay for β-TG (20) gave quantitatively identical reactions with β-TG, CTAP-III, and CTAP-III(A) as well as LA-PF-4 (9). While molecular weight data and immunological studies suggest that CTAP-III, CTAP-III(A), and β-TG are similar, biological activity studies show interesting differences. The biological activity of CTAP-III and CTAP-III(A) with respect to stimulation of synthesis of [^{14}C]hyaluronic acid and [^{3}H]DNA in human synovial cultures is recorded in Table 1. Dose–response curves for the DNA- and GAG-synthesis-stimulating activities of CTAP-III have been published and no inhibitory concentrations have been found (5, 6, 27). β-TG was without activity at all levels tested, including the higher concentration shown in Table 1.

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                          5                   10
CTAP-III      Asn-Leu-Ala-Lys-Gly-Lys-Glu-Glu-Ser-Leu-Asp-Ser-

CTAP-III(A)   Asn-Leu-Ala-Lys-Gly-Lys-Glu-Glu-Ser-Leu-Asp-Ser-

β-TG                      Gly-Lys-Glu-Glu-Ser-Leu-Asp-Ser-

                    15                  20
CTAP-III      Asp-Leu-Tyr-Ala-Glu-Leu-Arg-Cys-Met-Cys-Ile-

CTAP-III(A)   Asp-Leu-Tyr-Ala-Glu-Leu-Arg-Cys-Met-Cys-Ile-

β-TG          Asp-Leu-Tyr-Ala-Glu-Leu-Arg-Cys-Met-Cys-Ile-
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FIG. 3. Amino-terminal sequence of CTAP-III and CTAP-III(A). Reduced, alkylated protein (250 nmol) was submitted to automated Edman degradation. Resulting phenylthiohydantoin derivatives were quantitatively identified by HPLC or gas chromatography by using a norleucine phenylthiohydantoin standard. Repetitive yields were calculated to be 95.8%. The sequence of β-TG was determined by Begg *et al.* (12).

Amino Acid Sequence Studies. Sequence studies of CTAP-III and CTAP-III(A) were undertaken to clarify the relationship of these materials to β-TG and LA-PF-4. Amino-terminal sequence analyses of CTAP-III and CTAP-III(A) were carried out (Fig. 3), and these results indicate that the amino acid sequences of CTAP-III and CTAP-III(A) are identical in residues 1–23 and correspond to LA-PF-4 (9) in residues 1–12. CTAP-III differs from β-TG by the presence of an additional tetrapeptide (Asn-Leu-Ala-Lys) on the amino terminus of CTAP-III (8). To further define these relationships, CTAP-III and CTAP-III(A) were evaluated for their carboxyl-terminal residues by carboxypeptidase Y digestion (26) (Table 2). The terminal four residues (-Glu-Ser-Ala-Asp) were identical to those reported for β-TG (12). Two-dimensional chromatography/electrophoresis of trypsin-digested oxidized CTAP-III gave a peptide map identical to that of β-TG except for the detection of an additional cationic peptide, presumably representing the amino-terminal peptide (Fig. 4).

Present data show that CTAP-III and CTAP-III(A) are the same with respect to biologic activity, molecular weight, electrophoretic properties, reactivity with anti-CTAP-III antisera, peptide maps, and amino acid sequences 1–23 and 82–85. These characteristics are shared by LA-PF-4, except that less structural data is available concerning the latter protein. β-TG is like CTAP-III in these various characteristics except that it lacks biological activity, and lacks one peptide, the amino-terminal tetrapeptide. On the strength of these data, we propose that CTAP-III has the primary structure recorded in Fig. 5.

Three different laboratories have now isolated the same platelet protein [CTAP-III, CTAP-III(A), LA-PF-4] by three different methods, and it may be argued that it is unlikely that all three methods would yield biologic activities that reside in a contaminant undetected by physical or chemical means. To further assign the biologic activity of these platelet preparations to the protein that we have characterized, we undertook an immunologic experiment which shows that anti-CTAP-III IgG removes both CTAP-III and biologic activity from an appropriate

Table 2. Carboxyl-terminal residues of CTAP-III and CTAP-III(A)

Residue	Recovery, nmol	
	CTAP-III	CTAP-III(A)
Asp	42	21
Ala	37	15
Ser	25	8
Glu	14	5

Amino acids were determined by automated analysis and corrected for internal standards. CTAP-III and CTAP-III(A) (50 nmol each) were digested with carboxypeptidase Y for 45 min. Data show that identical amino acids were obtained for the two preparations. The deduced sequence is -Glu-Ser-Ala-Asp.

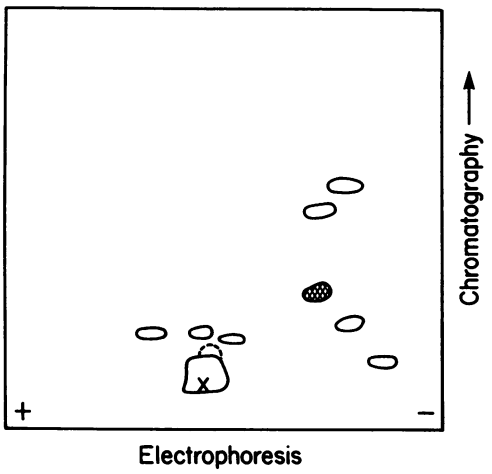

FIG. 4. Tryptic peptide maps. Peptide mixtures of CTAP-III or β-TG were applied at the origin (×). The peptide represented by the cross-hatched spot was present only in the CTAP-III digest; all other peptides were common to CTAP-III and β-TG.

incubation mixture. Table 3 shows that preimmune IgG does not deplete CTAP-III biologic activity, whereas anti-CTAP-III IgG forms a precipitate with a purified CTAP-III preparation; the supernatant is depleted of biologic activity and the immune precipitate retains biologic activity of the same general magnitude found in the control CTAP-III preparation. Because anti-CTAP-III was raised in rabbits against electrophoretically homogeneous CTAP-III, it seems unlikely that a second antibody (raised against a chemically undetected antigen) is responsible for removing biologic activators from the test preparation.

Table 3. Removal of CTAP-III mitogenic activity with anti-CTAP-III antibody

Agonist	IgG	[^{3}H]DNA synthesis, cpm/10^4 cells	Experimental/control	*P*
None	None	16,400 ± 1,900	—	—
None	Preimmune	32,000 ± 6,300	1.96	<0.005
None	Immune	10,000 ± 2,100	0.60	<0.005
CTAP-III	None	63,000 ± 1,900	3.85	<0.001
CTAP-III	Preimmune	88,000 ± 9,100	5.35	<0.001
CTAP-III	Immune (supernatant)	21,000 ± 3,500*	1.30	<0.05
CTAP-III	Immune (precipitate)	70,000 ± 1,900	4.25	<0.001

Preimmune and anti-CTAP-III IgG were isolated from staphylococcal protein A-Sepharose. Incubation mixtures consisted of 0.5 ml of vehicle or CTAP-III and 0.5 ml of preimmune or immune IgG; incubation was for 1 hr at 37°C and then 8 hr at 4°C. Supernatant and immune precipitate were separated by centrifugation, and the precipitate was resuspended in saline for the bioassay. CTAP-III was used at 11 μg/ml in the assay wells; preimmune and immune IgG were added at 354 and 1,216 μg/ml, respectively, in assay wells. Data are mean ± SD. *P* is for comparison with the [^{3}H]DNA synthesis by cells treated with saline vehicle alone.

* Mixture after immune precipitation was compared to CTAP-III without IgG; the marked reduction in stimulatory properties of the immune supernatant was significant ($P < 0.001$); the immune precipitate (containing CTAP-III) retained full stimulatory capability (last line of table).

DISCUSSION

A summary of selected biological characteristics of the better defined proteins isolated from platelets is presented in Table 4. Each is a low molecular weight protein secreted by activated platelets. They have alkaline isoelectric points (7.0–8.5) but differ biologically in their effects on DNA and GAG synthesis. Three of the four low-heparin-affinity proteins stimulate GAG synthesis; the same peptides [CTAP-III, CTAP-III(A), and LA-PF-4] stimulate DNA synthesis. β-TG and PF-4 are without effect on these metabolic characteristics.

Anti-CTAP-III IgG did not prevent CTAP-III stimulation of DNA synthesis in short-term experiments; whether it might protect against agonist degradation in longer studies would be of interest. LA-PF-4 isolated from the medium of thrombin-treated fresh platelets is a small protein with a reported isoelectric point of 8.0 that has antigenic determinants identical to β-TG and CTAP-III by immunodiffusion and radioimmunoassay (6, 7, 9). Studies in one of our laboratories (C.W.C.) showed that LA-PF-4 stimulated DNA and GAG synthesis (7). Partial amino acid sequence analysis (D.A.W.) showed that residues 5–12 of LA-PF-4 were identical to residues 1–8 of β-TG (9), and residues 1–4 were identical to the amino-terminal tetrapeptide of CTAP-III. The carboxyl-terminal sequence of LA-PF-4 has not been reported. It is likely that LA-PF-4, released from thrombin-treated platelets and isolated in a different manner than CTAP-III, is identical to CTAP-III isolated from the platelet mass itself.

It is possible that the methods employed in isolating CTAP-III and CTAP-III(A) account for the protection of the amino-terminal tetrapeptide and growth factor activity that are missing from β-TG. CTAP-III was extracted into acid/ethanol and CTAP-III(A) was processed via an early heat step, either of which might retard proteolytic digestion.

Many reports document the isolation of a "platelet-derived growth factor" (3–10, 29, 30). These isolated products have sim-

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                                5                   10                  15                  20
NH2-Asn-Leu-Ala-Lys-Gly-Lys-Glu-Glu-Ser-Leu-Asp-Ser-Asp-Leu-Tyr-Ala-Glu-Leu-Arg-Cys-

                                25                  30                  35                  40
Met-Cys-Ile-Lys-Thr-Thr-Ser-Gly-Ile-His-Pro-Lys-Asn-Ile-Gln-Ser-Leu-Glu-Val-Ile-

                                45                  50                  55                  60
Gly-Lys-Gly-Thr-His-Cys-Asn-Gln-Val-Glu-Val-Ile-Ala-Thr-Leu-Lys-Asp-Gly-Arg-Lys-

                                65                  70                  75                  80
Ile-Cys-Leu-Asp-Pro-Asp-Ala-Pro-Arg-Ile-Lys-Lys-Ile-Val-Gln-Lys-Lys-Leu-Ala-Gly

                                85
Asp-Glu-Ser-Ala-Asp-COOH
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FIG. 5. Proposed primary structure of CTAP-III. Residues 1–4 are unique to CTAP-III. Residues 5–23 were identified by direct sequence analysis of CTAP-III and are identical to residues 1–19 of β-TG as reported by Begg *et al.* (12). Residues 82–85 were identified by carboxypeptidase digestion and are identical to residues 78–81 of β-TG.

Table 4. Comparison of physiochemical properties of platelet proteins

Protein	Ref.	Platelet source	Antiheparin activity, units/mg	Molecular weight	Isoelectric point, pH	Amino-terminal residue	Stimulates DNA synthesis	Stimulates GAG synthesis
CTAP-III	6	Outdated	?	9,278*	8.5	Asn	Yes	Yes
CTAP-III(A)		Outdated	2	9,278*	8.0	Asn	Yes	Yes
LA-PF-4	9	Fresh	2	9,070†	8.0	Asn	Yes	Yes
β-TG	12	Outdated	?	8,851*	7.0	Gly	No	No
PF-4	13	Outdated	27	7,772*	7.6	Glu	No	?

* These molecular weights are calculated from the published primary structures.
† Molecular weight from sodium dodecyl sulfate/polyacrylamide gel electrophoresis.

ilarities, but sufficiently critical discrepancies remain to discourage regarding all as the same protein. Thus, it appears that CTAP-III differs from the factor of Heldin *et al.* (29) and Antoniades *et al.* (30) in molecular weight. Heldin *et al.* (29) reported the isolation of a disulfide-linked two-chained protein of molecular weight 31,000. On the basis of the total amino acid composition (29), it is conceivable that CTAP-III [CTAP-III(A), LA-PF-4] is derived from this larger protein. The possibility that CTAP-III is proteolytically regulated under physiologic conditions is enhanced by a recent report (31) demonstrating that LA-PF-4 [CTAP-III, CTAP-III(A)] can be proteolytically converted to β-TG by incubation with plasmin or trypsin with attenuation of biological activities.

It may be important to broaden the conventional view of "growth factors" as agents that are "mitogenic" and stimulate DNA synthesis. Clearly, regulatory molecules (such as CTAP-III) that stimulate formation of extracellular matrix GAG are contributing to "growth" irrespective of effects on DNA synthesis. Dose–response curves with CTAP-III show stimulation of DNA and GAG synthesis in monolayer cultures of nontransformed adult human connective tissue cells that is detectable at agonist concentrations of 100–250 ng/ml; marked stimulation occurs over the range of 1–20 μg/ml (5, 6). Whereas many regulatory agents stimulate both DNA and GAG synthesis, others are restricted to effects on GAG metabolism (32, 33). CTAP-III is apparently not even restricted to stimulating the anabolic aspect of cell function, because recent data show that it stimulates formation and secretion of plasminogen activator by human synovial cells (34).

Further studies are needed to elucidate the role of CTAP-III in platelet–vessel wall interactions during regulation of hemostasis and to clarify the role of CTAP-III in vasculitis, degenerative vascular disease, and inflammatory diseases in which plasma levels of the CTAP-III antigen are known to be markedly increased (35–37).

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