Cohn process influences the functional anticoagulant activity of human protein C

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ABSTRACT. Cohn fraction IV (CFIV) is a byproduct of a plasma fractionation process known as the Cohn process. It is an inexpensive source of protein C, retaining about 90% of protein C (PC) in human plasma. We investigated whether PC is affected during the Cohn process and evaluated correlations among coagulant activity, amidolytic activity and PC antigen during the Cohn process. CFIV was redissolved with citrate-buffered saline for 5 h at 4°C, and then centrifuged at 3500 g for 40 min at 4°C. Functional anticoagulant activity was measured with a one-stage coagulation method based on activated partial thromboplastin time. The functional amidolytic activity of PC was determined using chromogenic substrate assay, and measurement of PC antigen was performed by ELISA. In CFIV, anticoagulant activity declined significantly, with a loss of >80%, while amidolytic activity was not significantly altered, compared to PC antigen. Prior to the Cohn process, high-rank correlations were observed in cryosupernatant, with $r_s = 0.921$ for anticoagulant and amidolytic activities (P = 0.009), 0.896 for anticoagulant activity and antigen (P = 0.014) and 0.832 for

amidolytic activity and antigen (P = 0.031). After the Cohn process in CFIV, there was also a high correlation between amidolytic activity and antigen (r_s = 0.782, P = 0.038). There were no significant correlations between anticoagulant activity and antigen (r_s = 0.223, P = 0.653), or anticoagulant and amidolytic activity (r_s = 0.236, P = 0.675). We conclude that the Cohn process significantly influences the anticoagulant activity of PC. Compared to the antigen, PC lost greater than 80% of its anticoagulant activity, but retained its amidolytic activity, during the Cohn process.

Key words: Protein C; Anticoagulant activity; Amidolytic activity; Cohn process

INTRODUCTION

Human protein C (PC), a 62-kDa vitamin K-dependent zymogen of serine protease, is a glycoprotein mainly synthesized in the liver (Kisiel, 1979; Athanasiadis et al., 2011). Its structure is similar to other vitamin K-dependent proteins that affect blood clotting, such as factor II, VII, IX, and X. It is the pivotal anticoagulant and antithrombotic protein in the human coagulation cascade and regulates blood coagulation by inactivating activated factors V and VIII in the presence of calcium ions and phospholipids (Kisiel et al., 1977; Griffin et al., 1982; Esmons, 1989, 2012). In addition, PC has antiapoptic and anti-inflammatory properties, although the molecular mechanism responsible for these properties is not well-understood (Dettenmeier et al., 2003; Mosnier et al., 2007; Ní Ainle et al., 2011). Recent studies revealed that PC has neuroprotective effects and mediates new blood vessel formation and neurogenesis after controlled cortical impact (Petraglia et al., 2010). PC is a trace protein that is quite labile at an average concentration of 4 mg/L, with a half-life of 6 h in human blood (Esmon, 2012).

However, hereditary deficiency of PC can reduce the body's ability to prevent thrombosis as well as increase the risk of sepsis, deep vein thrombosis, and other clotting complications (Miletich et al., 1987; Hasstedt et al., 1998; Hoshi et al., 2007; Toussaint and Gerlach, 2009; Heleen van Ommen and Middeldorp, 2011; Brummel-Ziedins et al., 2012; Tang et al., 2012). Furthermore, acquired PC deficiency is frequently observed in meningococcemia, hepatopathy, and bone marrow transplant recipients. Currently, fresh frozen plasma, heparin, and Coumadin are used to treat PC deficiency and other hypercoagulable conditions (International Warfarin Pharmacogenetics et al., 2009). However, the therapeutics of fresh frozen plasma requires frequent infusions once or twice daily to achieve significant symptom improvement, which can lead to volume overload (Marlar et al., 1992) and increase the risk of blood-borne infections, such as human immunodeficiency virus, hepatitis B virus, and human parvovirus (Wu and Bruley, 2002). Two other methods, heparin and Coumadin, may cause harmful side effects, such as skin necrosis and excessive bleeding, which are life-threatening. Moreover, pregnant women cannot use Coumadin (Schramm, 1993).

Plasma-derived PC concentrates are efficient and display an excellent safety profile. They are generally well-tolerated and have no reported drug interactions, blood-borne infections, bleeding, or prothrombotic complications (Dreyfus et al., 2007; Kroiss and Albisetti, 2010). Until 2002, PC-related diseases have resulted in 300,000 hospitalizations and 50,000

deaths in the United States (Wu and Bruley, 2002). This places a substantial economic burden on the healthcare system (Wu and Bruley, 2002). The incidence rate of PC deficiency is 1 in 300 people, whereas the incidence of clinically significant PC deficiency is estimated to be 1 in 20,000 worldwide (Dahlbäck and Villoutreix, 2003). Therefore, therapeutic replacement of PC is a preferable alternative if large quantities of PC can be produced inexpensively.

Cohn fraction IV (CFIV), a byproduct of the Cohn process, often discarded, particularly in developing countries, retains over 90% of PC in the plasma and contains several homologous vitamin K-dependent proteins, such as factor II, VII, IX, and X, as well as other plasma proteins (Lee et al., 2008). The Cohn process, also known as cold ethanol fractionation, is a series of purification steps developed by Cohn et al. (1946) during World War II to separate various proteins from blood plasma. The process is based on the differential solubility of albumin and other plasma proteins that can be controlled by adjusting pH, ethanol concentration, temperature, ionic strength, and protein concentration (Cohn et al., 1946; Matejtschuk et al., 2000). Valuable coagulation factors located in cryoprecipitate, such as factor VIII and von Willebrand factor, are removed by continuous flow centrifugation before the Cohn process. Subsequently, fibrinogen, immunoglobulins, and albumin are sequentially precipitated (Cohn et al., 1946; Kistler and Nitschmann, 1962; Matejtschuk et al., 2000).

We previously successfully purified PC from CFIV (Wang et al., 2010). During the purification of PC from CFIV, we found that the anticoagulant activity of PC was not correlated with its antigen and amidolytic activity. We hypothesized that the Cohn process influences the functional activity of PC. In this study, we investigated whether PC is affected during the Cohn process and evaluated the correlation between anticoagulant activity, amidolytic activity, and antigen of PC during the Cohn process.

MATERIAL AND METHODS

Pretreatment of samples

Six random batches of samples were provided by Guizhou Taibang Biological Products Co., Ltd. (Guiyang, China) for this study. For each batch, approximately 2500 L fresh frozen plasma was thawed at 1-6°C and the cryoprecipitate was removed by centrifugation. The cryosupernatant (CSP) was used to prepare various therapeutic proteins using the Cohn process. CSP, supernatant I (SI), supernatant II+III (SII+III), and CFIV, numbered from 1-4, respectively (Figure 1), were selected for the experiments. CFIV was re-dissolved in citrate-buffered saline in a ratio of 1:10 (w/v) for 5 h at 4°C, and then centrifuged at 3500 g for 40 min at 4°C. The supernatant was used in subsequent experiments.

Anticoagulant activity assay

The anticoagulant activity of PC was determined in a one-stage coagulation method based on activated partial thromboplastin time (aPTT). First, 70 μL diluted sample and 70 μL PC deficient plasma (American Diagnostica Inc., Stamford, CT, USA) were mixed and incubated at 37°C for 2 min. Next, equal amounts aPTT regents (Chengdu Union Biotechnology Co., Ltd., Chengdu, China) and PC activator, Protac® (Hyphen Biomed, Neuville-sur-Oise, France) were added. After another incubation for 5 min, calcium chloride was added and clotting was initiated. A direct relationship was observed between clotting time and PC anticoagu-

lant activity. The anticoagulant activity of PC was converted using a calibration curve derived from a plasma calibrator (Hyphen Biomed) and the corresponding clotting time.
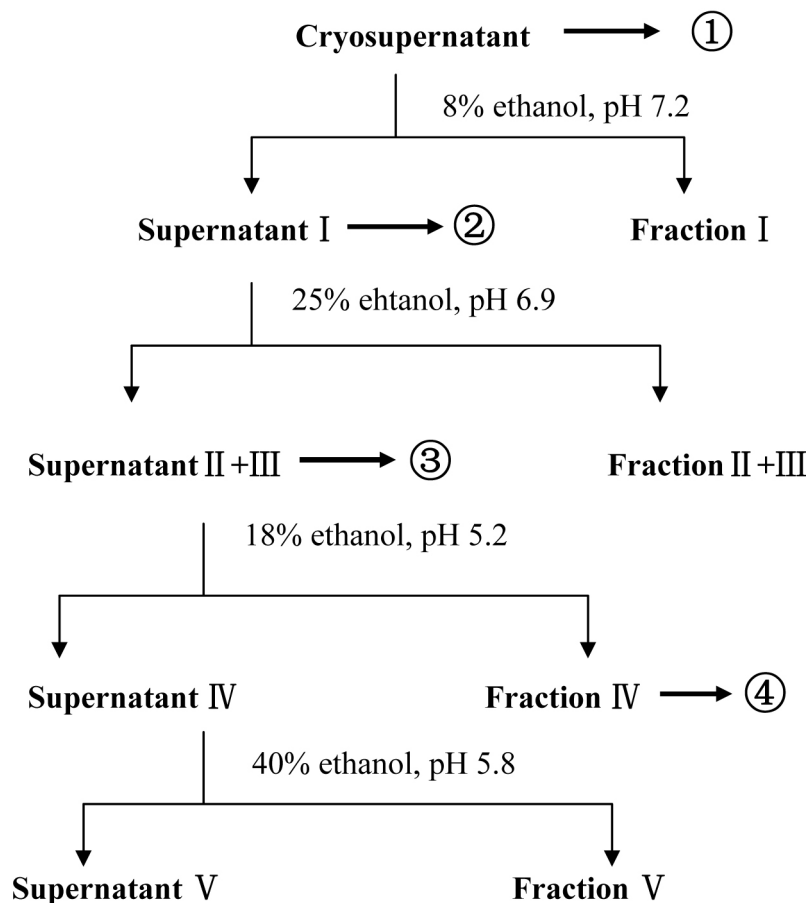
Cryosupernatant ⟶ ①

8% ethanol, pH 7.2

Supernatant Ⅰ ⟶ ②   **Fraction** Ⅰ

25% ehtanol, pH 6.9

Supernatant Ⅱ+Ⅲ ⟶ ③   **Fraction** Ⅱ+Ⅲ

18% ethanol, pH 5.2

Supernatant Ⅳ   **Fraction** Ⅳ ⟶ ④

40% ethanol, pH 5.8

Supernatant Ⅴ   **Fraction** Ⅴ

Figure 1. Flow diagram of the Cohn process. The samples numbered from 1 to 4 were used for the experiment. The 6 batches of cryosupernatant were 2470, 2480, 2460, 2460, 2470, and 2470 L, respectively. The data were 2700, 2710, 2690, 2690, 2700, and 2700 L for supernatant I, 4020, 4040, 4010, 4030, 4020, and 4030 L for supernatant II+III, and 182, 194, 186, 196, 184, and 188 kg for Cohn fraction IV. The supernatant of redissolved Cohn fraction IV was 1820, 1940, 1860, 1960, 1840, and 1880 L, respectively.

Amidolytic activity assay

The amidolytic activity of PC was measured using a chromogenic substrate assay. To 96-well flat-bottom microtiter plates, we added 25 μL plasma calibrator (Hyphen Biomed) in different dilution ratios and 75 μL Protac® (Hyphen Biomed) and incubated the plate at 37°C for 8 min to rapidly catalyze the conversion of PC into the active form, activated protein C. Subsequently, 75 μL CS-21(66) (Hyphen Biomed), the specific chromogenic substrate, was added and the mixture was incubated at 37°C for 10 min. Hydrolysis was terminated by adding 75 μL 50% glacial acetic acid. Activated protein C specifically cleaves the substrate and re-

leases para-nitroaniline. The color was measured at 405 nm using SpectraMax M2e (Molecular Devices, Sunnyvale, CA, USA). For the endpoint method, the calibration curve was obtained by plotting on the abscissae the amidolytic activity of PC in the plasma calibrator with different dilutions and on the ordinate, the corresponding absorbance. PC amidolytic activities in the tested samples were directly calculated according to the calibration curve.

Antigen content assay

The antigen content of PC was quantified using an enzyme-linked immunosorbent assay (ELISA). A kit for this test was purchased from Hyphen Biomed. Diluted test samples were introduced into microwells pre-coated with a rabbit polyclonal antibody specific for human protein C. After incubation for 1 h at room temperature, the wells were washed 5 times. Next, horseradish peroxidase-conjugated anti-(H)-PC immunoconjugate (a rabbit polyclonal antibody specific to human protein C) was added to the microwells. After washing, the peroxidase substrate, tetramethylbenzidine in the presence of hydrogen peroxide, was introduced. After incubation for 5 min at room temperature, the reaction was stopped with 0.45 M sulfuric acid. The absorbance was measured at 450 nm (reference absorbance: 620 nm) using Spectra-Max M2e. The absorption value was directly proportional to the antigen content of PC in the tested samples.

Statistical analysis

All experiments were carried out in triplicate. Data was analyzed to determine the group mean, with error bars reported as the standard deviation (SD). The Shapiro-Wilk test was to determine the normality of the distribution. Multi-group comparisons (anticoagulant activity, amidolytic activity, and antigen of PC in each step of the Cohn process) were performed using one-way analysis of variance followed by the Tukey multiple means comparison test. Comparisons of the same PC parameter in the different steps were accomplished using two-tailed, paired Student t-tests. Correlations among anticoagulant activity, amidolytic activity, and antigen in the 4 steps were computed using Spearman's rank correlation analysis. A P value <0.05 was considered to be statistically significant. In addition, statistical analyses were conducted using the SPSS statistics software, version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

To simplify the measurement data, the antigen content of PC was converted to functional activity based on the theoretical value of 1 IU functional activity in 1 mL normal pooled plasma, which is equivalent to 4 µg antigen of PC.

Mean levels and range of PC in samples from different steps during the Cohn process are shown in Table 1. There were no significant changes (<10%; P ≥ 0.064) from CSP to SI. However, there was an acute decrease of approximately 34% from SI to SII+III (P < 0.001). Anticoagulant activity, amidolytic activity, and antigen content were decreased by 88.4 (P < 0.001), 48.2 (P < 0.001), and 48.0% (P < 0.001), respectively, during CFIV compared to SII+III. PC levels continuously decreased from CSP to CFIV (P < 0.001), with a total decline of 93.1, 66.7, and 67.8% for anticoagulant activity, amidolytic activity, and antigen content, respectively.

Table 1. Anticoagulant activity, amidolytic activity, and antigen content of PC in CSP, SI, SII+III, and CFIV.

Analyte	CSP	SI	Changes (%)	P*	SII+III	Changes (%)	P*	CFIV	Changes (%)	Total changes (%)§	P*
Anticoagulant activity (10⁶ IU)	2.17 ± 0.10 (2.04-2.27)	1.96 ± 0.27 (1.52-2.22)	9.7	0.064	1.29 ± 0.15 (1.13-1.45)	34.2	<0.0001	0.15 ± 0.03 (0.12-0.20)	88.4	93.1	<0.0001
Amidolytic activity (10⁶ IU)	2.16 ± 0.10 (2.03-2.28)	2.10 ± 0.26 (1.83-2.46)	2.8	0.483	1.39 ± 0.18 (1.23-1.65)	33.8	<0.0001	0.72 ± 0.05 (0.66-0.80)	48.2	66.7	<0.0001
Antigen content (10⁶ IU)	2.42 ± 0.10 (2.26-2.54)	2.29 ± 0.32 (1.84-2.63)	5.4	0.269	1.50 ± 0.15 (1.34-1.69)	34.5	<0.0001	0.78 ± 0.07 (0.75-0.87)	48.0	67.7	<0.0001

N = 6 samples. Data are reported as means ± SD, followed by the range of the results in parentheses. Mean change in PC levels in that step *vs* the step of the previous measurement. Total change in PC levels from CSP to CFIV. *PC levels in that step are compared with that in CSP, using two-tailed, paired Student *t*-tests. Antigen content was converted to functional activity based on the theoretical value that there is 1 IU functional activity in 1 mL normal pooled plasma, equivalent to 4 μg antigen of PC.

Figure 2 shows the total functional activity and antigen content of PC in the 4 steps of the Cohn process. In CSP, the anticoagulant activity, amidolytic activity, and antigen content of PC were similar, and the ratio of the 3 parameters was 1.0:1.0:1.1. Similarly, no significant difference was observed in SI (1.0:1.1:1.2) and SII+III (1.0:1.1:1.2). However, we observed significantly lower anticoagulant activity, approximately 90% less than amidolytic activity and antigen content in CFIV, with the ratio of 1.0:5.1:5.5 (Figure 2; P < 0.001). There were no significant differences in amidolytic activity and antigen in CFIV.
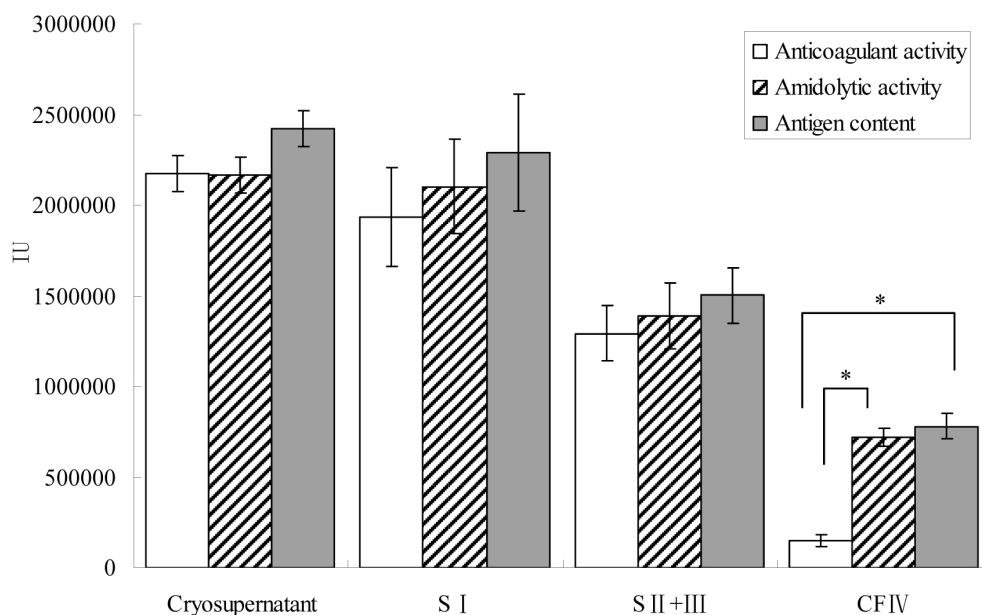

Figure 2. Anticoagulant activity, amidolytic activity and antigen of PC in CSP, SI, SII+III, and CFIV. Antigen content was converted to functional activity based on the theoretical value in 1 mL normal pooled plasma containing 1 IU functional activity and 4 μg antigen of PC. The statistical significances of anticoagulation activity, amidolytic activity, and antigen content of PC in the different steps of the Cohn process were evaluated, by one-way analysis of variance followed by the Tukey multiple comparison test. *P < 0.001.

Spearman's rank correlations among anticoagulant activity, amidolytic activity, and antigen in the 4 steps are summarized in Table 2. Before the Cohn process, in CSP, Spearman's rank correlation coefficients of the 3 PC parameters were high, with $r_s = 0.921$ for anticoagulant *vs* amidolytic activities, 0.896 for anticoagulant activity *vs* antigen, and 0.832 for amidolytic activity *vs* antigen ($P < 0.05$ for all comparisons). During the Cohn process, good correlations were observed for the 3 levels in SI and SII+III (the lowest $r_s = 0.858$; $P < 0.05$). After the Cohn process, CFIV showed good correlation between amidolytic activity and antigen ($r_s = 0.782$; $P = 0.039$). However, no obvious correlation was found for anticoagulant activity *vs* antigen ($r_s = 0.223$; $P = 0.653$) or anticoagulant *vs* amidolytic activity ($r_s = 0.236$; $P = 0.675$).

Table 2. Rank correlations among anticoagulant activity, amidolytic activity, and antigen of PC in the 4 steps.

Analyte	CSP		SI		SII+III		CFIV	
	r_s	P value*	r_s	P value*	r_s	P value*	r_s	P value*
Anticoagulant activity *vs* amidolytic activity	0.949	0.003	0.858	0.021	0.864	0.019	0.143	0.787
Anticoagulant activity *vs* antigen content	0.845	0.034	0.906	0.013	0.943	0.004	0.2	0.704
Amidolytic activity *vs* antigen content	0.886	0.019	0.867	0.015	0.881	0.02	0.782	0.038

r_s = Spearman's rank correlation coefficient. *Rank correlation coefficients were performed using the Spearman rank correlation test.

DISCUSSION

We attempted to purify PC from CFIV because it retains most of the PC in the plasma and therefore improves utilization of plasma. The byproduct CFIV is typically discarded by most plasma fractionators, but Bayer Corporation (Clayton, NC, USA) successfully purified α1-antitrypsin from Cohn fraction IV-1 and obtained large profits (Chen et al., 1998). Lee et al. (2007, 2008) and Rezania et al. (2007) demonstrated that it is feasible to purify PC from Cohn fraction IV-1. We previously successfully purified PC from CFIV (Wang et al., 2010). More importantly, the use of CFIV as the starting material does not interfere with the main fractionation of albumin. Although the PC concentrate may be contaminated with blood-borne viruses, only quarantined plasma has been used for this process in China. The plasma is stored frozen for at least 90 days until the donor returns and retests as negative for human immunodeficiency virus, heptatitis B virus, and hepatitis C virus. Virus removal (i.e., nanofiltration) and inactivation techniques (i.e., solvent/detergent treatment, pasteurization), robust methods widely used in the blood plasma industries, are also performed (Horowitz et al., 2004).

PC is a trace but extremely critical protein with anticoagulant, antiapoptic, and anti-inflammatory properties in human blood plasma. There is little data regarding the PC levels retained in CFIV. Lee et al. (2007, 2008) reported that about 90% PC in plasma was retained in Cohn fraction IV-1. However, we observed that only approximately 32% PC in the initial material (CSP) was retained in CFIV. These discrepancies may have resulted from variations in the Cohn process.

The Cohn process is a milestone in the field of blood fractionation and it has been significantly modified over the past 60 years (Lane and Vallet, 1984; Matejtschuk et al., 2000). However, the Cohn process has not been thoroughly evaluated. Furthermore, the conventional process has not been modernized by manufacturing companies. Regardless, the process still

serves as a cornerstone for plasma fractionation; its influence can be observed in the development of newer methods, such as ion exchange chromatography for intravenous immunoglobulin (Burnouf, 2007). Although there are some disadvantages to this method, such as high pollution and no modernization, the Cohn process is universal and practical for use by pharmacological and medical industries. There are more than 10 process variations but only 2 main processes, the original Cohn process and the Kistler and Nitschmann variation, are commonly used (Matejtschuk et al., 2000). In the original Cohn process, as displayed in Table 3, the conditions for fraction I were 8% ethanol, pH 7.2, and -3°C, and those for fraction II+III were 25% ethanol, pH 6.9, and -5°C. Fraction IV-1 was precipitated by decreasing the ethanol concentration from 25 to 18% and decreasing the pH from 6.9 to 5.2. Fraction IV-4 was obtained by raising the ethanol concentration to 40% and the pH to 5.8. For fraction V, the pH was reduced to 4.8 while the ethanol concentration was maintained at 40% (Cohn et al., 1946). In the Kistler and Nitschmann variation, the precipitate A, which is equivalent to CFII+III, was precipitated at an ethanol concentration of 19%, pH 5.85, and -3°C. The precipitate B, also known as fraction IV, was precipitated in 40% ethanol, pH 5.85, and -8°C. Similarly to the original Cohn process, fraction V (precipitate C) was obtained at 40% ethanol and pH 4.8 (Table 3) (Kistler and Nitschmann, 1962). In general, 2 steps of the original Cohn process (Cohn fractions IV-1 and IV-4) were combined into 1 step (CFIV) in the Kistler and Nitschmann method. Currently, the original Cohn process is used by most American manufacturers, whereas the cost-effective and independent Kistler and Nitschmann variation has been widely accepted by many European and Asian fractionators (Matejtschuk et al., 2000). Therefore, Cohn fractions IV-1 and IV-4 are obtained in the United States, while CFIV is obtained in Asia and Europe using the different Cohn process variations. This may explain why PC levels in CFIV in the current study differed from those generated by the Cohn fraction IV-1 by Lee et al. (2008).

Table 3. Differences between the original Cohn process and Kistler and Nitschmann variation.

	Original Cohn process					Kistler and Nitschmann variation		
	Fraction I	Fraction I+III	Fraction IV-1	Fraction IV-4	Fraction V	Precipitate A (Fraction II+III)[#]	Precipitate B (Fraction IV)[#]	Precipitate C (Fraction V)[#]
Ethanol (%)	8	25	18	40	40	19	40	40
pH	7.2	6.9	5.2	5.8	4.8	5.85	5.85	4.8
Temperature (°C)	-3	-5	-5	-5	-5	-3	-8	-8

Data are from Cohn et al. (1946) and from Kistler and Nitschmann (1962). [#]Corresponding denomination of the original Cohn process.

This study was carried out to assess the influence of the Cohn process on PC activity. The theoretical ratio of anticoagulant activity, amidolytic activity, and antigen content of PC in plasma is 1:1:1. We found that the ratio was 1:1:1 in CSP, which was identical in SI (1:1:1) and SII+III (1:1:1) during the Cohn process. Our results for CSP, SI, and SII+III agreed with the theoretical values. Interestingly, the anticoagulant activity of PC was significantly lower when compared to amidolytic activity and antigen content (1:5.1:5.5; P < 0.001) in CFIV, while the ratio of amidolytic activity to antigen content of PC (1:1.1) was consistent with the theoretical value. Similarly, Spearman's rank correlation coefficients among anticoagulant activity, amidolytic activity, and antigen did not change significantly for CSP, SI, and SII+III, with the lowest $r_s = 0.832$ (P < 0.05). After the Cohn process, a significant correlation between

amidolytic activity and antigen (r_s = 0.782, P = 0.039) was observed in CFIV. However, correlations for anticoagulant activity *vs* antigen (r_s = 0.223, P = 0.653) and for anticoagulant *vs* amidolytic activity (r_s = 0.236, P = 0.675) were significantly decreased. This indicates that in CFIV, anticoagulant activity significantly differed from amidolytic activity and antigen content. Thus, PC lost most of its anticoagulant activity but retained its amidolytic activity during the Cohn process.

Aggregate analysis of the anticoagulant and amidolytic properties of PC, as demonstrated through mutagenesis studies by several research groups (Sun et al., 2003; Kerschen et al., 2007; Mosnier et al., 2009; Nicolaes et al., 2010), revealed the different structure-function facets of PC. Numerous PC variants have been designed and assayed for their functional properties, and it appears that special surface areas denote different functional properties. To better understand the relationship between the structure and function of PC, Wildhagen et al. (2011) collected the available structure-function data of PC and created a perspective of the 3-D structure of PC. Mutagenesis studies have demonstrated the importance of a number of amino acid residues located in the Gla-domain of PC, such as residues Phe4, Leu5 and 8, Arg9 and 15, Glu6, 7, 16, 20, and 26, and His10, for PC anticoagulant activity (Wildhagen et al., 2011). Suitable Ca^{2+}-dependent formation is important for maintaining the overall structure of the Gla-domain, which is essential for PC anticoagulant activity. Similarly, residues in the 37-loop (Lys191-193), Ca^{2+}-binding loop (Arg229-230), and autolysis loop (Glu309) of the serine protease domain are important for anticoagulant function by means of cleavage of activated factors V and VIII (Wildhagen et al., 2011). Furthermore, Asp71 in the epidermal growth factor domain plays a pivotal role in the interaction of PC with protein S and Ca^{2+}. Site-direct mutagenesis studies indicate that PC will lose its anticoagulant function completely or partially, whereas its amidolytic activity is unaffected if one or more of the described residues are mutated. Anticoagulant and amidolytic activities simultaneously decrease when residue Ser336 in the 214-loop of the serine protease domain or Ser360 in the active site is mutated (Wildhagen et al., 2011). Collectively, the anticoagulant activity of PC may be affected by more critical residues (more than 30 variants) than the amidolytic activity (only several mutants). During the Cohn process, residues in the Gla-domain, 37-loop, Ca^{2+}-binding loop, or autolysis loop may be damaged. These residues are critical for maintaining the Ca^{2+}-dependent conformation of the Gla-domain, binding proteins to phospholipid, and inactivating activated factors V and VIII, which are essential elements for PC anticoagulant activity. Additionally, residues in the 214-loop and active site, which are essential for both anticoagulant and amidolytic activities, may not be affected. These results suggest that PC significantly loses its anticoagulant activity but retains its amidolytic activity during the Cohn process.

In summary, we found that compared with the PC antigen, anticoagulant activity was significantly affected with a more than 80% loss, while the amidolytic activity was relatively unaffected during the Cohn process. These results support the hypothesis that the Cohn process influences the anticoagulant activity of PC. Furthermore, in the Cohn process, only approximately 30% of PC in the plasma was retained in CFIV.

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