The adsorption of blood coagulation factors II, VII, IX and X from human plasma to aluminium flydroxide

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The Adsorption of Blood Coagulation Factors II, VII, IX and X from Human Plasma to Aluminium Hydroxide

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Introduction

Nearly all methods designed for the isolation of the factors of the so-called prothrombin complex from blood plasma use the adsorption of the coagulation factors onto an insoluble inorganic substance as a first step. The extensive use of barium sulphate for this purpose has induced a number of investigators to try and find optimum conditions for the adsorption and the desorption, both the selective removal of contaminating proteins and the completest possible elution of the coagulation factors (1, 2, 3, 4, 5, 6). When citrated plasma is the starting material for prothrombin isolation, barium sulphate cannot be used and one has to employ the barium citrate technique (7) or to use aluminium hydroxide. An inquiry into the adsorption properties of aluminium hydroxide towards rabbit prothrombin and the elution of the adsorbed protein by means of phosphate has been made (8).

For more than 30 years already go the efforts of Seegers to purify prothrombin and to elucidate its role in the process of blood coagulation. The view of coagulation as advocated by him is based upon the observations that activities capable of converting prothrombin into thrombin could arise from apparently homogeneous preparations of prothrombin itself. He and his school have devised a system of nomenclature based on this finding. Prothrombin in the sense as used by Seegers contains the coagulation activities called factor II and X in the International Committee on Hemostasis and Thrombosis nomenclature and probably also factors VII and IX.

The purification procedure developed by Seegers (22) is based upon euglobulin precipitation from diluted acidified bovine plasma and on magnesium hydroxide adsorption. The prothrombin obtained is further purified by ammonium sulphate precipitation, isoelectric precipitation and adsorption with barium carbonate. These prothrombin preparations appeared to be homogeneous by a number of criteria such as ultracentrifugal analysis and N-terminal analysis (23).

When prothrombin, as defined by Seegers, indeed is the parent molecule of both factor IIa and factor Xa, then one can expect the ratio of factor II and factor X activities to remain constant during adsorption and salting out procedures, in other words, the adsorption to be essentially unselective towards these two activities. There is therefore a well founded basic interest in determining whether or not adsorption and salting out procedures are selective towards the factors of the prothrombin complex.

Factors II, VII, IX, and X can be adsorbed from citrated human plasma to aluminium hydroxide. The aim of this work was to investigate whether the adsorption of factors II, VII, IX, and X is selective, whether selective desorption is possible and

what conditions are optimal for both processes. Moreover, the highest possible purification was striven after. This was done by varying a number of physico-chemical parameters playing a role.

Materials and Methods

Chemicals

Unless otherwise stated, chemicals were of analytical grade and were obtained from Merck.

Aluminium hydroxide

Aluminium hydroxide "moist gel" BDH, batch nr. 0340600 was used. 200 g of the paste were homogenized in distilled water by means of a Blendor to give 1 litre of suspension.

This is referred to as 20% aluminium hydroxide suspension. If necessary, dilutions were made of it with distilled water. The pH of the suspension was 7.0.

Veronal acetate buffer

As described in ref. 9.

ACD-plasma

440 ml of blood is collected in a glass bottle containing 60 ml of a solution that is 0.140 M in disodium citrate and 0.183 M in glucose. Centrifugation is done for 30 min at 750 g. The plasma obtained is not platelet-free. ACD-plasma of 20 donors was pooled and frozen at –20° C.

Determination of coagulation factor activities

Factor II was assayed with a reagent and a method described by Koller, Loeliger and Duckert (12), and Loeliger and Koller (13).

Factor VII reagent and factor X reagent were prepared according to Hemker et al. (9).

Factors VII and X were assayed by a method described in the same reference.

Factor IX was assayed by the method of Veltkamp et al. (14), using congenitally factor IX deficient plasma as a reagent.

Execution of adsorption and elution experiments

All experiments were carried out in polycarbonate centrifuge tubes (Servall). The centrifuge used was a Servall SS 4, unrefrigerated, but placed in the cold room at +4° C. If necessary, the centrifuge was used at room temperature. To an amount of plasma in a centrifuge tube, aluminium hydroxide suspension was added, mostly one tenth of the plasma volume and of such concentration as to give the desired final quantity. The concentration of the adsorbent will be expressed as grams of adsorbent added to 1 litre of plasma, abbreviated as g/l.pl. or alternatively as mg/ml.pl.

After the adsorption period, chosen for an individual experiment, the adsorbent was precipitated by centrifugation at 20.000 g for 5 min. If the extent of adsorption was to be determined, the residual activity in the supernatant plasma was assayed. In elution experiments the sediment was resuspended in the eluant, mostly one quarter of the plasma volume. After a certain lapse of time, the adsorbent was sedimented by centrifugation at 20.000 g for 5 min and the eluate was tested for activity.

Eluates were never dialysed before analysis but dilutions as high as possible were chosen when the eluting substance interfered with the coagulation test. In case of comparison of different concentrations of eluant, each of the eluates was diluted in such a way that the eluant concentration in the test system was the same. Dilutions were made with Veronal-acetate buffer.

Results

The Effect of the pH and the Concentration of the Adsorbent

At five different pH values: 5,4; 6.4; 7.4; 8.4; and 9.4 ACD plasma was adsorbed by aluminium hydroxide in four different concentrations, 1, 2, 3, and 4 g/l plasma. The plasma was brought to the desired pH value by slow addition of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide under vigorous stirring. The adsorbent was added in one-tenth plasma volume. The adsorption period was 5 min. The supernatant after centrifugation was adjusted to pH 7.4, and tested for residual activity.

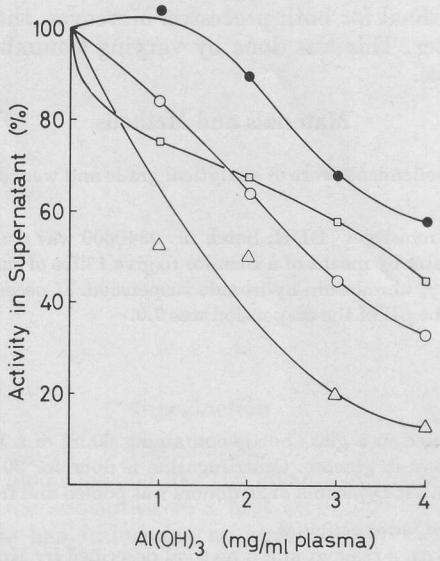

Fig. 1. The adsorption of blood coagulation factors II, VII, IX, and X from human plasma to different concentrations of aluminium hydroxide at pH 5.4. ○———○ factor II, ●———● factor VII, △———△ factor IX, □———□ factor X.

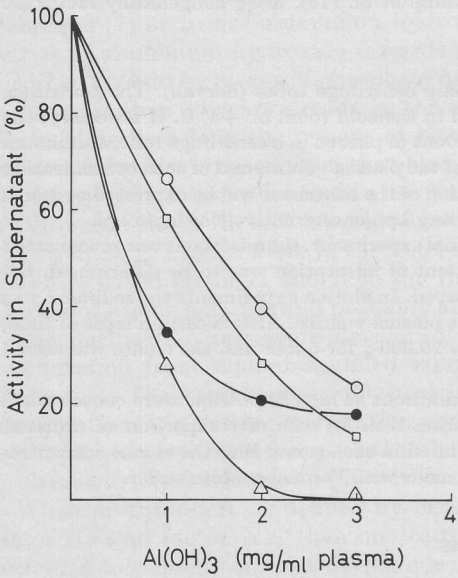

Fig. 2.

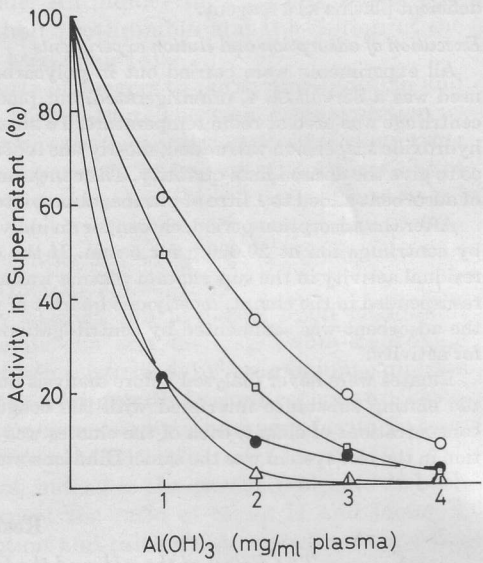

Fig. 3.

Fig. 2. The adsorption of blood coagulation factors II, VII, IX, and X from human plasma to different concentrations of aluminium hydroxide at pH 6.4. ○———○ factor II, ●———● factor VII, △———△ factor IX, □———□ factor X.

Fig. 3. The adsorption of blood coagulation factors II, VII, IX, and X from human plasma to different concentrations of aluminium hydroxide at pH 7.4. ○———○ factor II, ●———● factor VII, △———△ factor IX, □———□ factor X.

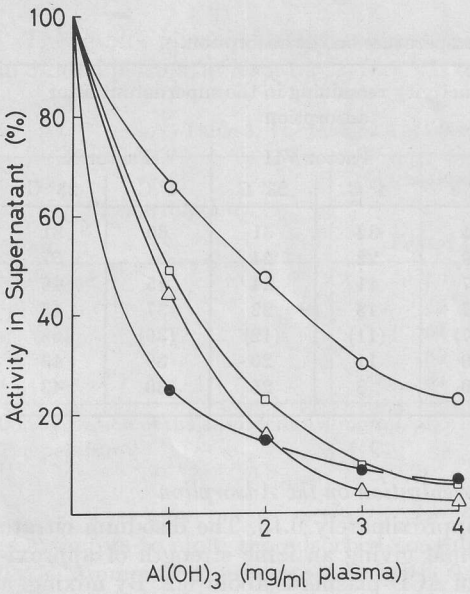

Fig. 4.

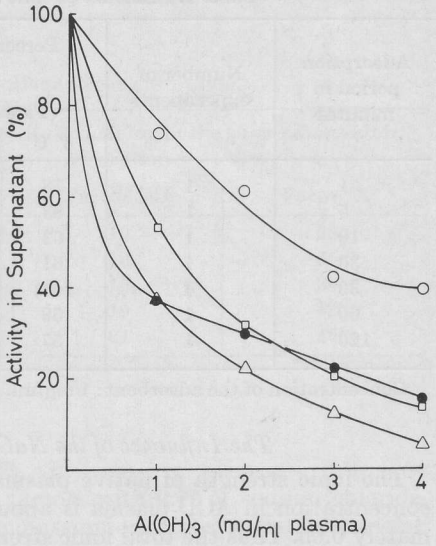

Fig. 5.

Fig. 4. The adsorption of blood coagulation factors II, VII, IX, and X from human plasma to different concentrations of aluminium hydroxide at pH 8.4. ○———○ factor II, ●———● factor VII, △———△ factor IX, □———□ factor X.

Fig. 5. The adsorption of blood coagulation factors II, VII, IX, and X from human plasma to different concentrations of aluminium hydroxide at pH 9.4. ○———○ factor II, ●———● factor VII, △———△ factor IX, □———□ factor X.

All experiments were done at 20° C. At each pH, a blank received water instead of adsorbent. After neutralization to pH 7.4, its activity was taken as 100%. These controls indicated that low and high pH did not inactivate the coagulation factors in the lapse of time necessary for the experiment. Figs. 1–5 show the results. For all factors, adsorption is optimal at neutral pH.

Factor II is least strongly adsorbed, followed by factor X, whereas factors VII and IX have the highest affinity for the adsorbent. At pH 5.4 the adsorption of factor VII is greatly reduced. At pH values near neutral 5 g aluminium hydroxide/l.pl. is sufficient for complete adsorption of the factors. Higher concentrations will only adsorb more contaminating proteins.

Influence of Time and Temperature on the Adsorption

To 30 ml ACD-plasma was added 1.5 ml of 2% aluminium hydroxide suspension to give a final concentration of 1 mg/ml.pl. Immediately after adding the adsorbent and after 5, 10, 30, 60, and 120 min 5 ml of the mixture was centrifuged. The supernatants were assayed for activity .The experiment was done at +4° C and at +23° C. Table 1 gives the results. At both temperatures, most of the adsorption takes place in the first minutes. At lower temperature, it takes longer time to reach adsorption equilibrium and the adsorption seems to be more effective too. To check this, the experiment with 30 min adsorption period was repeated four times. The mean of the results obtained is incorporated in Table 1, placed in brackets. The values suggest that only the adsorption of factor II is more complete at low temperature.

Table 1. Influence of time and temperature on the adsorption.

Adsorption period in minutes	Number of experiments	Percentage activity remaining in the supernatant after adsorption					
		Factor II		Factor VII		Factor X	
		4° C	23° C	4° C	23° C	4° C	23° C
1	1	78	84	32	51	60	81
5	1	63	69	22	24	51	57
10	1	63	67	21	24	45	46
30	1	61	67	13	22	37	45
30	4	(61)	(67)	(11)	(12)	(36)	(38)
60	1	58	69	11	20	30	43
120	1	57	70	8	26	30	43

Concentration of the adsorbent: 1 mg/ml. pl.

The Influence of the NaCl Concentration on the Adsorption

The ionic strength of native plasma is approximately 0.15. The disodium citrate concentration in ACD-plasma is about 17 mM giving an ionic strength of approximately 0.05. Thus the total ionic strength of ACD-plasma is about 0.2. By mixing, a series of different ionic strength values was obtained at a constant plasma dilution of four times. The concentration of the adsorbent was 1 mg/ml.pl. The adsorption period was 5 min. After centrifugation, the supernatants were brought to ionic strength 0.15 by dilution with water or addition of 1 M sodium chloride before being tested for activity. Blanks in which the adsorbent suspension had been replaced by distilled water showed that the temporal increase or decrease of the ionic strength had no demonstrable effect on the activity of the coagulation factors. Table 2 gives the percentages of activity, found in the supernatants after adsorption. They are the means of a number of experiments. Increased ionic strength favours the adsorption whereas decreased ionic strength hampers it.

Table 2. The influence of the ionic strength on the adsorption.

Ionic strength	Number of experiments	Percentage of activity that remains in the supernatant after adsorption			
		Factor II	Factor VII	Factor IX	Factor X
0.05	2	97	69	93	97
0.09	3	75	51	80	74
0.16	3	69	41	58	60
0.31	3	56	31	48	48

Concentration of the adsorbent: 1 mg/ml. pl.
Adsorption period: 5 min
Temperature: +4° C
The figures for factor IX are the results of only one experiment.

The Influence of Plasma Dilution on the Adsorption

ACD plasma was diluted with 0.15 M sodium chloride to give a series of dilutions. Adsorption was carried out with 1 mg of adsorbent/ml.pl. The temperature was +4° C. The supernatants after centrifugation were diluted before analysis in such a way that all dilutions had the same citrate and protein concentration.

The results, given in Table 3, suggest that the adsorption is less complete when done in diluted plasma, at least for factors VII and X.

Table 3. The influence of plasma dilutions on the adsorption.

Plasma dilution	Percentage of activity remaining in the supernatant after adsorption		
	Factor II	Factor VII	Factor X
Undiluted	50	10	28
1,5 ×	49	15	35
2 ×	51	15	38
3 ×	53	19	43
4 ×	54	21	46

Concentration of the adsorbent: 1 mg/ml. pl.
Temperature: +4° C.

The Elution

Attempts to elute the adsorbed coagulation factors with 0.15 M sodium chloride, 0.2 M ammonium sulphate and with 0.1 M potassium oxalate were unsucessful. EDTA and citrate, excellent eluants for clotting factors adsorbed to barium sulphate proved to be capable to elute much protein from aluminium hydroxide but only a few percent of the adsorbed clotting factors. Sodium EDTA 0.3 M pH 8.0 and sodium citrate 0.2 M pH 8.0 were used in these experiments. Phosphate is very effective in eluting the coagulation factors from aluminium hydroxide. These observations lead to the conclusion that it is advantageous to wash the adsorbent with 0.3 M sodium EDTA pH 8.0 and/or 0.2 M sodium citrate pH 8.0, before eluting the coagulation factors with phosphate. A purer phosphate eluate results.

The Influence of the pH of the Phosphate Buffer on the Elution

20 ml of ACD plasma were adsorbed for 5 minutes by 5 mg aluminium hydroxide/ml.pl. at room temperature. The pellets obtained after centrifugation were eluted for 15 min with 5 ml portions of sodium potassium phosphate buffer 0.2 M at different pH values. Table 4 gives the yields of the coagulation factors in the respective eluates. It is evident that higher pH leads to more complete elution. At pH 6.0, poor yields were obtained. Although pH 9.0 seems to give the best results, it was decided to work at pH 8.0 in subsequent experiments.

Table 4. The influence of the pH of the phosphate buffers on the elution.

pH of eluting phosphate buffer	Percentage yield of the coagulation factors in the eluate			
	Factor II	Factor VII	Factor IX	Factor X
7.0	42	34	75	29
7.5	47	49	80	35
8.0	42	42	94	36
8.5	52	52	106	37
9.0	53	53	108	38

Phosphate concentration: 0.2 M
Elution period: 15 min
Temperature: room temperature.

The Influence of the Molarity of the Phosphate Buffer on the Elution

20 ml aliquots of ACD-plasma were adsorbed with 100 mg of aluminium hydroxide for 5 min, and centrifuged. The pellets were eluted for 15 min at room temperature, with 5 ml portions of phosphate buffers, having a pH of 8.0, but different molarity. Table 5 gives the yield of the coagulation factors in the eluates. Optimal results were obtained with buffers having a molarity between 0.25 and 0.30. When elution is done in the cold room, a higher concentration than 0.25 M cannot be employed because disodium hydrogen phosphate crystallizes out rapidly then. On the basis of these experiments elution is routinely done with 0.25 M phosphate pH 8.0. Low phosphate concentrations elute relatively much factor II. When elution is done in a column by means of a phosphate gradient of increasing molarity, factor II is the first to appear in the effluent.

Table 5. The influence of the molarity of the phosphate buffer on the elution.

Molarity of the eluting phosphate buffer	Percentage yield of the coagulation factors in the eluate			
	Factor II	Factor VII	Factor IX	Factor X
0.025	48	32	29	22
0.050	48	40	38	25
0.100	66	43	63	52
0.150	68	48	74	54
0.200	66	50	76	54
0.250	74	57	100	54
0.300	66	74	120	43
0.500	60	65	81	38

pH of eluting buffer: 8.0
Elution period: 15 min
Elution temperature: room temperature.

The Influence of the Ionic Strength on the Elution

The observation that the effectiveness of phosphate as an eluant increases with its concentration suggests that the ionic strength plays an important role. It was attempted to elute the coagulation factors with a low phosphate concentration but at a high ionic strength, brought about by addition of salts, which, by themselves, are completely ineffective. 0.036 M phosphate +0.6 M sodium chloride and 0.036 M phosphate +0.2 M ammonium sulphate were chosen and were compared with 0.25 M phosphate. The pH of these solutions was 8.0 and the ionic strength was 0.7. The results are given in Table 6. When comparing them with those listed in Table 5, it is clear that addition of sodium chloride to the phosphate does not promote elution but rather has an adverse effect. Admixture of ammonium sulphate on the other hand greatly enhances the eluting capacity of phosphate. The type of ion is thus of decisive importance.

The Influence of the Duration of the Elution

Four 30 ml aliquots of ACD plasma were adsorbed with 5 mg aluminium hydroxide/ml.pl. for 5 min at 4° C and centrifuged. Each of the sediments was eluted with 3 ml 0.25 M phosphate buffer pH 8.0 at 4° C. Elution was allowed to take place for 3, 7, 12, and 30 min respectively. Table 7 gives the results. Most of the activity is eluted within the first 3 min but a longer elution period leads to a better yield.

Table 6. The influence of the ionic strength on the elution.

Composition of the eluant	Percentage yield of the coagulation factors in the eluate		
	Factor II	Factor VII	Factor X
0.25 M phosphate	72	58	63
0.036 M phosphate + 0.6 M sodiumchloride	8	13	7
0.036 M phosphate + 0.2 M ammoniumsulphate	60	55	58

pH of the eluants: 8.0
Ionic strength of the eluants: 0.7
Elution period: 15 min
Elution temperature: room temperature.

Table 7. The influence of the duration of the elution.

Elution period in min	Percentage yield of the coagulation factors in the eluate		
	Factor II	Factor VII	Factor X
3	56	82	50
7	60	86	45
12	68	100	49
30	72	100	52

Eluant: 0.25 M phosphate pH 8.0
Elution temperature: 4° C

Table 8. Denaturation of the coagulation factors in the adsorbed state.

Adsorption period in min	Percentage yield of the coagulation factors in the eluate			
	Factor II	Factor VII	Factor IX	Factor X
5	68	55	53	37
25	60	48	53	37
45	55	48	38	28
90	53	48	40	28
150	45	45	32	22
210	38	38	20	13

Eluant: 0.25 M phosphate pH 8.0
Elution period: 15 min
Elution temperature: room temperature

Denaturation of the Coagulation Factors in the Adsorbed State

Aliquots of ACD plasma were adsorbed with 5 mg adsorbent/ml.pl. at room temperature for 5, 25, 45, 90, 150 and 210 min respectively, and centrifuged. Elution was done with half the plasma volume of 0.25 M phosphate buffer pH 8.0 for 15 min at room temperature.

Table 8 shows the results obtained. It is evident that the amount of activity that can be eluted from the adsorbent becomes smaller the longer the factors have been in

the adsorbed state. This experiment was repeated at 4° C and 37° C with three different adsorption periods, 5, 60, and 240 min. The results of this comparison are shown in Table 9. The denaturation is temperature-dependent.

Table 9. Denaturation of the coagulation factors in the adsorbed state.

Adsorption period in min	Percentage yield of the coagulation factors in the eluates							
	Factor II		Factor VII		Factor IX		Factor X	
	4° C	37° C	4° C	37° C	4° C	37° C	4° C	37° C
5	73	54	113	41	43	23	82	53
60	65	27	90	17	30	12	78	24
240	35	10	58	4	15	4	47	14

Preparative Isolation of the Factors of the Prothrombin Complex

Based on the foregoing experiments, we devised the following working scheme for the optimal preparative isolation of factors II, VII, IX and X from human citrate plasma.

To ACD-plasma, add aluminium hydroxide suspension 200 g/l, to a final concentration of 5 g/l. Stir for 5 min. Centrifuge at 3000 × g for 10 min. Resuspend the sediment in one tenth plasma volume 0.3 M sodium EDTA pH 8.0 and stir vigorously for 10 min. Centrifuge at 3000 × g for 10 min. Wash the sediment in the same way with 0.1 M sodium citrate pH 8.0. Resuspend the sediment in one twentieth plasma volume 0.25 M potassium phosphate pH 8.0, stir vigorously for 10 min, and centrifuge as mentioned above. Repeat the elution with an equal volume of phosphate buffer. Pool the phosphate eluates. This preparation is called PPBS. The whole isolation procedure takes about 3 h. Table 10 shows the results.

Table 10. Characteristics of five different batches of PPSB.

	Activity with respect to starting plasma	Yield with respect to starting plasma	Purification with respect to starting plasma
Factor II	420% (350 – 500%)	42% (35 – 50%)	121 × (92 – 145 ×)
Factor VII	1690% (1550 – 1800%)	169% (155 – 180%)	481 × (410 – 531 ×)
Factor IX	1030% (650 – 1500%)	103% (65 – 150%)	296 × (188 – 480 ×)
Factor X	470% (350 – 540%)	47% (35 – 54%)	133 × (103 – 156 ×)

Mean values are given.
The extremes are placed between brackets.

Discussion

Investigations on the adsorption of proteins to adsorbents mostly can have hardly any theoretical foundation because of the complexity of the structures and their interactions; therefore, the approach is condemned to be essentially empirical in nature. Such is the situation with the adsorption of the coagulation factors. The adsorption of the factors of the so called prothrombin complex to barium sulphate and their subsequent elution has received considerable attention (1, 2, 3, 4, 5, 6) and on the basis of the result obtained it has been possible to create a picture of the mechanism of the adsorption and the elution (2, 5, 6).

The adsorption of the factors of the prothrombin complex to aluminium hydroxide has been studied less extensively. Only Munro and Munro (8) published a rather thorough study on the adsorption of rabbit prothrombin and its elution with phosphate. Before the start of the present investigation some data on the adsorption of human prothrombin to aluminium hydroxide were known to us (15).

The influence of the pH on the adsorption is complex because the pH affects both the properties of the adsorbent and those of the proteins. In the entire range of pH that has been studied here, the coagulation factors carry a negative charge because their isoelectric points are between 4 and 5. At neutral pH the aluminium hydroxide gel most probably carries a positive charge. Therefore, the existence of an electrostatic attractive force between the proteins and the adsorbent at neutral pH is almost sure. At more acid pH the negative charge on the coagulation factors is smaller. The electrostatic attraction would be expected to be decreased then and indeed a strong decrease in adsorption is observed, especially for factor VII. This has been seen in the case of barium sulphate too (3, 6). At high pH values the adsorption is also less effective, probably because the adsorbent looses its positive charge and the hydroxyl ions compete with the protein molecules for binding sites.

The adsorption of the coagulation factors does not proceed proportionally with the adsorbent concentration, indicating that proteins other than the coagulation factors compete for binding sites on the adsorbent; the more pronounced, the lower the concentrations of the factors have become. Low adsorbent concentrations do not remove the factors quantitatively from plasma, but give far purer preparations on elution, much potentially contaminating protein remaining unadsorbed. A comparable reasoning applies to the selectivity of the adsorption. The relative concentrations of the factors having changed by selective adsorption, the lowered concentrations of the more tightly bound factors counteract the advantage of their higher affinity for the adsorbent. This tends to make the adsorption less selective. Factors VII and IX are bound most firmly, followed by factor X, whereas factor II has the lowest affinity. Factor II also binds least strongly, to bentonite (16), to barium sulphate (17, 18), to asbestos (19), to aluminium oxide, and to magnesium carbonate (3).

It is reasonable to expect that the adsorption equilibrium is reached more quickly at high temperature and that the equilibrium shifts to the side of stronger adsorption with decreasing temperature. Indeed it was found that adsorption takes place more quickly at high temperature but it was observed that the extent of adsorption did not change with temperature, except perhaps for factor II, but the difference is small and it can easily be made a matter of debate if this represents a significant effect. Similar results have been obtained with the adsorption of factor VII to barium sulphate (4). Not always could the influence of temperature be demonstrated.

The statement that an increase in the ionic strength of the plasma leads to better adsorption would be too general. The experiments in which the effectiveness of a number of salts as eluants was investigated clearly demonstrate the specificity of the interactions between the salts and the adsorbent.

Therefore, the results presented in Table 2 only warrant the conclusion that increased sodium chloride concentration promotes the adsorption. In view of this it should be noted that the experiments on the influence of the ionic strength on the elution even suggest that chloride has an adverse effect on the elution. The observed diminished adsorption of the coagulation factors in case of dilution of the plasma with 0.15 M sodium chloride is difficult to interprete, because the effect is probably not caused by dilution alone. The ionic strength of the plasma is higher than that of the diluting solution, and the relative concentration of citrate and chloride is not the same in each

of the dilutions. The conclusion can only be practical: dilution as done in this experiment is of no advantage.

Elution of adsorbed proteins can often be brought about by slightly alkaline solutions of salts having polyvalent anions, like ammonium sulphate, ammonium carbonate, sodium citrate and especially phosphates (20).

In our experiments 0.15 M sodium chloride and 0.2 M ammonium sulphate were completely ineffective. Sodium EDTA 0.3 M pH 8.0 and sodium citrate 0.2 M pH 8.0 both desorb much protein from aluminium hydroxide but only small amounts of coagulation factors. EDTA and citrate have been found to be very good eluants for barium sulphate (2). Much inert protein but little coagulation factors can be eluted from this adsorbent by dicarboxylic acids, oxalate, malonate etc. (2), but we found 0.1 M potassium oxalate to be in capable of eluting protein from aluminium hydroxide. Phosphate is the eluant of choice for this adsorbent. Slightly alkaline pH values from 8 to 9 are to be preferred and a concentration between 0.25 and 0.30 M gives optimal yields. These results confirm those reported in the literature (8). A combination of phosphate and ammonium sulphate sometimes constitutes a very effective eluant (20). This proved to be true in our system too.

0.036 M phosphate + 0.2 M ammonium sulphate gave results almost as good as those obtained with 0.25 M phosphate. In contrast to general experience (21) the time necessary for elution is short. Long elution times are of limited advantage only. The coagulation factors are rather labile in the adsorbed state. The denaturation is strongly temperature-dependent and proceeds rapidly. Therefore, it is of utmost importance to work as quickly as possible and at low temperature.

When the adsorbent is washed with EDTA and citrate before the elution with phosphate, these time consuming washes lead to a purer product but the price paid for it is more extensive denaturation and therefore poorer yields.

It will be clear from the experiments reported that a reversible adsorption shows a certain selectivity between these factors.

Especially the difference between factors II and X is of theoretical interest. The differences are small but readily reproducible in sufficiently precise experiments. They infer that it is not justified to come to the conclusion that the biological activities of the factors II and X reside in one and the same molecule when this conclusion is based on a purification procedure using $Al(OH)_3$ as an adsorbent. Presumably this conclusion can be extended to the use of any substance permitting adsorption and desorption of coagulation factors.

Summary

The influence of pH, adsorption time, ionic strength, buffer type and plasma concentration on adsorption and elution of the factors of the prothrombin complex from normal human ACD plasma onto $Al(OH)_3$ is investigated. Reproducible small differences are found between the behaviour of the four factors II, VII, IX, and X.

Résumé

On étudie l'influence du pH, du temps d'adsorption, de la force ionique, du type de tampon et de la concentration du plasma sur l'adsorption et l'élution des facteurs, du complexe prothrombinique à partir du plasma humain ACD avec l'hydroxyde d'aluminium. Des petites différences entre le comportement des facteurs II, VII, IX et X se laissent démontrer.

Zusammenfassung

Es wurde der Einfluß des pH, der Absorptionszeit, der Ionenstärke, der Art des Puffers und der Plasmakonzentration auf die Adsorption und Elution der Faktoren des Prothrombinkomplexes aus normalem menschlichem ACD-Plasma durch Aluminiumhydroxyd untersucht. Es wurden reproduzierbare geringe Differenzen im Verhalten der vier Faktoren II, VII, IX und X beobachtet.

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