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Studies on the Coagulation of Chicken Blood

X. Adsorption Analysis of the $\varkappa$- and δ-Factor Activities

ØYVIND SØRBYE*

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

No heterogeneity could be detected by adsorption analysis of the $\varkappa$- and δ-factor activities of oxalated plasmas using a series of crystalline adsorbents. The experiments support the idea that each of these two activities represents the activity of a single coagulation factor.

The minimum concentration of an adsorbent required for complete adsorption of either of the two activities is proportional to the activity of the corresponding factor and represents an absolute value for its concentration in plasma.

Mixtures of vitamin K-deficient and coumachlor (or dicumarol) plasmas have been introduced as substrates for differentiation and assay of three different coagulation activities in normal plasma (the $\varkappa$-, δ-, and φ-factors [1]). The method permitted studies on the adsorption of each of the three activities from oxalated normal plasmas. Widely different relative adsorption capacities for the three activities were observed in preliminary experiments using a series of crystalline adsorbents [2]. These results indicated that slight differences in the physico-chemical properties of the factors responsible for the three activities could be revealed by adsorption studies. It was pointed out that adsorption by crystalline adsorbents might be a technique of general value for analysis and differentiation of the coagulation activities lacking in artificial substrates, and for a preliminary characterization of the corresponding coagulation factors. Thus, subsequent detailed studies on the adsorption of φ-factor activity from normal plasmas disclosed that this activity was not homogenous [3]. The presence of five different factors with φ-factor activity was indicated by adsorption experiments when chicken brain thromboplastin was used as assay accelerator. Three of these factors proved inactive when thromboplastin was replaced by Russell's viper venom (RVV)-cephalin, and the φ-factor activity measured with RVV-cephalin was shown to represent the combined activity of two different φ-factors.

* Fellow of the Royal Norwegian Council for Scientific and Industrial Research.

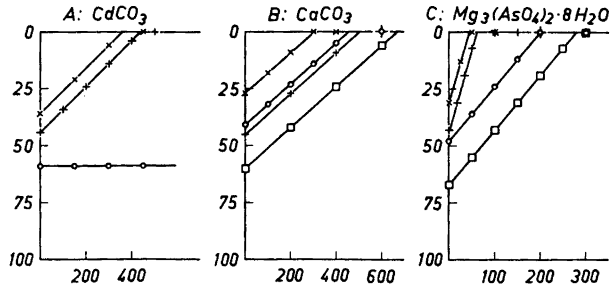

Fig. 1.

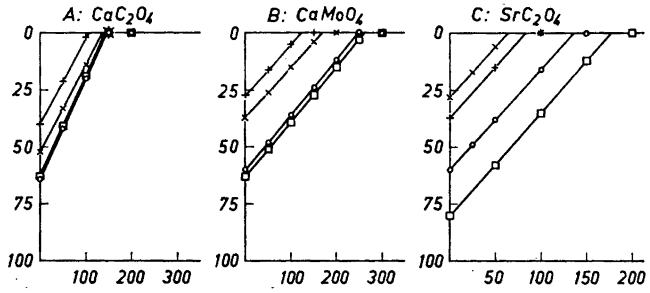

Fig. 2.

Figs. 1—4 (A—C). Adsorption of the $\varkappa$-factor and δ-factor activities.

Ordinates: $\varkappa$-factor or δ-factor activity levels (in % of previously observed maximal levels). Abscissae: adsorbent in mg/ml.

In the present study detailed adsorption analysis was applied to test the homogeneity of the $\varkappa$- and δ-factor activities of normal plasmas.

MATERIALS AND METHODS

The materials and methods used in this study were as described previously [1-3]. $\varkappa$- and δ-Factor activities of untreated and adsorbed samples of oxalated plasma were determined simultaneously as described [1], using chicken brain thromboplastin as assay accelerator. Adsorbed activity was determined as the difference between original activity and activity remaining after adsorption of plasma aliquots by graded amounts of the adsorbents.

RESULTS AND DISCUSSION

The $\varkappa$-factor and δ-factor activity levels of the plasmas used in the present study varied considerably. The $\varkappa$-factor level of chicken plasma has been shown to depend on a simultaneous provision of six unidentified dietary factors in addition to vitamin K [4]. A satisfactory diet for maintenance of a high and

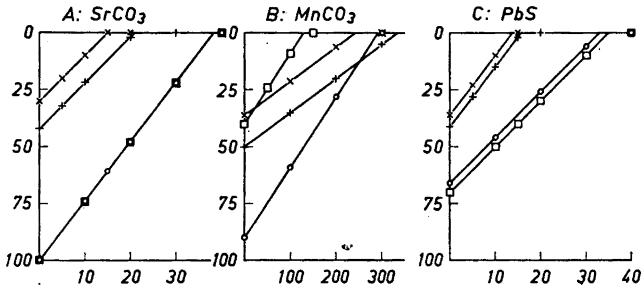

Fig. 3.

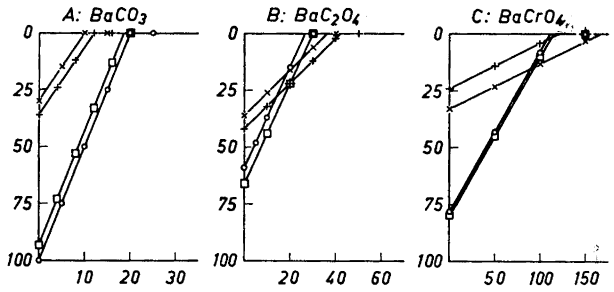

Fig. 4.

—+— and —×— : δ-factor activity.
—□— and —○— : κ-factor activity.
—+— and —□— : plasma A.
—×— and —○— : plasma B.

constant level of δ-factor in chicken plasma has not yet been worked out, and the dietary dependence of δ-factor is, at present, an unsolved problem. The adsorption of κ-factor activity could accordingly be studied in plasmas with κ-factor levels varying between 40 and 100 % of maximal, while the δ-factor levels of the same plasmas varied only between 24 and 52 % of the levels observed occasionally in previous experiments [1,2].

Representative results obtained by use of twelve different adsorbents are presented in Figs. 1—4 (A—C). With the exception of cadmium carbonate (Fig. 1A), which gave no detectable adsorption of κ-factor activity, complete adsorption of the κ- and δ-factor activities could be accomplished with all the adsorbents tested. Single-sectioned rectilinear adsorption curves were obtained in all experiments, and accordingly, adsorbed κ-factor or δ-factor activity was proportional to the concentration of the particular adsorbent until complete adsorption of the respective activities. In this respect the two activities differ characteristically from the φ-factor activity measured by chicken brain thromboplastin ($\varphi_{tpl.}$-factor activity). Only parts of this activity

could be adsorbed by calcium carbonate and magnesium arsenate, and with the other adsorbents two-, three-, or four-sectioned rectilinear adsorption curves were obtained [3]. The latter curves were interpreted as independent adsorption of different coagulation factors with similar effects in the test system employed. This interpretation is supported indirectly by the present experiments in which no corresponding adsorption heterogeneity could be demonstrated for the $\varkappa$-factor or δ-factor activity. Considering the capacity of the adsorbents to distinguish between different φ-factors (and also between different labile factors [5]), the curves obtained for adsorption of the $\varkappa$-factor and δ-factor activities strongly support our previous assumptions [1] that each of these two activities is associated with a single coagulation factor.

The results of different experiments, using the same adsorbent and plasmas with different or identical levels of $\varkappa$-factor and/or δ-factor, appeared as parallel or identical adsorption curves, respectively. Variations in $\varkappa$- or in δ-factor activity are thus reflected in proportional variations in the minimum concentration of an adsorbent required for complete adsorption of the factor. The capacity of each adsorbent for adsorption of $\varkappa$-factor or δ-factor activity appeared to be unrelated to the plasma concentration of the factors. This is in agreement with the interpretation of previous experiments [2,3], that the δ- and $\varkappa$-factors are adsorbed independently by the adsorbents, *i.e.*, by different adsorption "sites", the density and relative number of which may vary with different adsorbents and, to some extent, also with different batches of the same adsorbent [2].

The $\varkappa$- and δ-factor concentrations of plasmas may accordingly be expressed in absolute terms as the minimum concentration of a standard preparation of an adsorbent required for complete adsorption of the particular factor. This is in analogy with the proposed adsorption method for determination of the φ_2-factor concentration of plasma [3]. With the preparations used in this study, a strontium carbonate concentration of 38 mg/ml and a barium carbonate concentration of 20 mg/ml appeared to represent the maximum level of $\varkappa$-factor in chicken plasma. A determination of the corresponding values for maximum δ-factor level must await a successful unravelling of the dietary dependence of this factor.

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