PREPARATIVE FRACTIONATION OF SOLUBLE HUMAN LEUKOCYTE ANTIGENS BY ADSORPTION AND ION-EXCHANGE CHROMATOGRAPHY

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The development of effective methods of preparative fractionation of protein mixtures provides a basis for the creation of methods of isolation of antigens in a pure form [2]. Ion-exchange and adsorption methods of chromatography possess the highest resolving power for protein fractionation, and their use in "columnless" versions (using an eluent in a fixed concentration) enables expensive equipment to be dispensed with and work to be done with small volumes of biological material (protein mixtures) for fractionation [2].

Evaluation of the state of leukocyte function is an essential preliminary to analysis of the cellular component of immune homeostasis [1]. The study of leukocyte function at the molecular level by identifying and investigating individual substances synthesized by these cells is an important task at the present time. In particular, the present writers have described a system of human soluble leukocyte antigens (SLA), whose parallel immunochemical testing in hemolysates provides an accurate method of assessing leukocyte function [3]. The development of effective preparative methods of obtaining purified individual SLA, required for research into the determination of their biological function and also, perhaps, for immunocorrection, is becoming an extremely important task.

In the investigation described below the possibility of using adsorption and ion-exchange chromatography (with fixed concentration of the eluent) for preparative fractionation of individual SLA was studied.

EXPERIMENTAL METHOD

The technique of obtaining lysed leukocytes from healthy human venous blood and from an extract of pus by freezing and thawing was described by the writers previously [3]. Experiments on adsorption of SLA on particular adsorbents and elution were carried out under the control of standard monspecific test systems [4] and individual SLA [3]. Because the content of some SLA in the hemolysate (SLA-5, SLA-6, SLA-8) was too small for chromatographic fractionation, an extract of pus, obtained during opening of an abscess, which had a high concentrationof these SLA, was used additionally.

Ion-exchange Sephadexes SP-C-50 and QAE-A-50 (from Pharmacia Fine Chemicals, Sweden), and also L 5/40 silical-gel and L 5/40 alumina (from Chemapol, Czechoslovakia) were used.

All sorbents were soaked beforehand in distilled water, the excess of which was removed by centrifugation at 1000 g for 5 min, and incubated with the original biological material (lysed leukocytes, extract of pus) with continuous mixing, in the ratio of 1:1 for 30 min. After incubation, unbound products were removed by washing five times with 10 volumes of water. The adsorbed SLA were eluted from silica-gel, kaolin, barium sulfate, and alumina in parallel tests in glass containers with mixing for 20 min, using four different eluents in fixed concentrations, which were added to the wet adsorbents in the ratio of 2:1 [2 M NaCl, 0.4 M Na_2HPO_4, 1 M $(NH_4)_2SO_4$, 1 M Na_2CO_3]. To elute SLA from ion-exchange Sephadexes only the 2 M NaCl solution was used. The eluates were separated from the adsorbents by centrifugation at 2000 g for 10 min. The eluates thus obtained, and also the supernatants after incubation of the biological material with adsorbents, were subjected to immunochemical analysis for nine individual SLA and ballast serum proteins, which were determined with the aid of a polyvalent antiserum against normal human plasma.

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TABLE 1. Adsorption and Elution of SLA in Adsorption and Ion-Exchange Chromatography

SLA	Silica-gel					Kaolin					Alumina					Barium sulfate					QAE-A-50		SP-C-50	
	A	B				A	B				A	B				A	B				A	B	A	B
		I	II	III	IV		I	II	III	IV		I	II	III	IV		I	II	III	IV		I		I
1	−					+	−	−	−	−	−					+	±	±	±	±	−		+	+
2	−					+	−	±	−	+	+	−	+	±	+	+	±	+	+	+	−		+	+
3	+	−	−	−	+	+	−	−	−	−	+	±	+	±	+	+	±	±	±	±	−		+	+
4	−					+	−	±	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	
5	+	−	−	−	±	+	−	−	±	±	+	−	−	−	−	−					−		+	+
6	+	−	−	−	±	+	−	−	−	−	+	−	−	±	±	−					−		+	+
7	+	−	−	−	−						+	−	+	+	+	−					+	+	−	
8	+	−	−	−	+	+	±	±	±	±	+	±	±	±	±	−					−		+	+
9	−					+	−	−	+	−	+	−	+	+	±	−					−		−	

Legend. A) Adsorption, B) elution. I) 2M NaCl, II) 0.4M Na_2HPO_4, III) 1M $(NH_4)_2SO_4$, IV) 1M Na_2CO_3. ±) Incomplete elution from sorbent.

EXPERIMENTAL RESULTS

A great difference was found both in the adsorption capacity of the adsorbents used and in the effectiveness of elution by means of the various eluents used (Table 1). For instance, on silica-gel, five of the nine SLA (SLA-3, SLA-5, SLA-6, SLA-7, and SLA-8) were adsorbed, of which only four were eluted by 1 M Na_2CO_3; complete desorption, moreover, was recorded only for SLA-3 and SLA-8. The use of NaCl, Na_2HPO_4, and $(NH_4)_2SO_4$ as eluents for all adsorbed SLA proved ineffective, but several ballast serum proteins could be desorbed with the aid of these eluents. Of the nine SLA tested, kaolin adsorbed eight (except SLA-7), but complete elution was discovered only for SLA-2, by means of 1 M Na_2CO_3 (Table 1). Alumina adsorbed seven SLA, and complete elution was possible for four of them: SLA-2 was completely eluted by Na_2HPO_4 and Na_2CO_3 as also was SLA-3, SLA-7 was completely desorbed by three eluents (Table 1), and SLA-9 was completely eluted by Na_2HPO_4 and $(NH_4)_2SO_4$.

Of the nine SLA only four were adsorbed on Ba_2SO_4; of this number, moreover, only SLA-2 could be completely eluted. Of the nine SLA only SLA-4 and SLA-7, which were completely eluted by 2 M NaCl, were adsorbed on the anion-exchange Sephadex QAE-A-50, and six SLA were adsorbed on cation-exchange Sephadex SP-C-50, and completely eluted. As might be expected, those SLA were adsorbed on the cation-exchange resin (Sephadex-SP-C-50) were not adsorbed on the anion-exchange Sephadex QAE-A-50, and vice versa (Table 1). Only SLA-9 was not adsorbed on either of the ion-exchange Sephadexes.

The use of the four above-mentioned eluents enabled the adsorbed proteins to be fractionated with greater resolving power than the use of the ionic strength gradient of any single eluting agent. When an adsorbed antigen for isolation is not removed by any eluent, it is best to carry out additional washing of the adsorbent with this solution for preliminary elution of the contaminating proteins. Incidentally, all the sorbents used in this version of chromatography possess high sorptive capacity for antigens, and it is unnecessary to use any complex system of columns.

The results can be used to obtain semipurified preparations of individual SLA and they occupy an important place in the development of schemes for obtaining highly purified preparations of these antigens. For example, a combination of adsorption chromatography on silica-gel [with preliminary washing with 1 M $(NH_4)_2SO_4$ and 0.4 M Na_2HPO_4 solutions to remove ballast proteins], and elution with 1 M Na_2CO_3, and ion-exchange chromatography on Sephadex SP-C-50 can already yield an "immunochemically pure" preparation of SLA-3

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