Recovery of Antibody Combining Activity by Interaction of Different Peptide Chains Isolated from Purified Horse Antitoxins

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Received January 5, 1963

Recently γ-globulin molecules have been found to consist of several peptide chains (Edelman, 1959; Franěk, 1961). After partial separation these chains were shown to belong to two types differing in molecular weight (Edelman & Poulik, 1961) and were named H (heavy) and L (light) chains (Edelman & Benacerraf, 1962). In different guinea pig antihapten antibodies several distinct L chains were found in starch gel electrophoresis (Edelman *et al.* 1961). A hypothetical explanation was given by these authors that the L chains contain the antibody active site or at least determine the specificity. Porter (1962) separated the H and L chains from partially reduced and alkylated rabbit antibody with the aid of Sephadex G—75. Unlike the mentioned hypothesis, Porter found the antibody activity in the peak of incompletely separated H chains. The aim of the present investigation was to estimate whether the antibody combining activity is determined by one (H or L) or more peptide chains.

Diphtheria and tetanus antitoxins were isolated from hyperimmune horse plasma by means of specific binding with corresponding anatoxins fixed on fine particles of cellulose (immunosorbents) (Gourvich, Ispolatovskaya & Myasoedova, 1961; Gourvich, Kusovleva & Tumanova, 1961; Nezlin *et al.* 1961). The same immunosorbents served also for the determination of antibodies and their fragments. The reaction of the antibodies with the immunosorbent, the washing and the elution with glycine buffer of pH 2.2 was carried out in centrifuge tubes. On an average, 65% of the purified diphtheria antitoxin reacted with the immunosorbent under the conditions used. Nonspecific adsorption on the immunosorbent was negligible in all cases (e.g. 0.3% of the total protein or less).

Cleavage of the reactive disulphide bonds was carried out in the following manner*: The antitoxin eluted from the immunosorbent was transferred into 0.5 M ammonium chloride on a column of Sephadex G-25. This solution was made 0.15 molar with respect to sodium sulphite and the pH adjusted to 8.6 with ammonia. Immediately, cupric nitrate in ammonia buffer of pH 8.6 was added to the final concentration 0.005 M. The mixture was allowed to stand for 5 hours at 20° C, then the protein was freed from non-volatile low molecular substances on a column of Sephadex G-25 in 0.25 M ammonium carbonate and freeze dried. S-sulpho diphtheria antitoxin contains still 25—30% protein reacting specifically with the immunosorbent. The same relative amount reacted in 0.6 M or 1.2 M urea in neutral pH. When the S-sulpho antitoxin was dissolved in a suitable medium for separation of peptide chains,

*) Franěk F.: Manuscript in preparation.

i.e. in 0.05 M formic acid with 6 M urea (pH ~ 3)*) and left in that medium 24 hours, the amount of protein reacting with the immunosorbent after neutralization and dilution with 4 or 9 volumes of 0.07 M sodium chloride decreased to a level of 3—5% of total protein. This is, however, an amount still possible to detect after separation of peptide chains.

Separation of antibody peptide chains was carried out in 0.05 M formic acid with 6 M urea on Sephadex G-100*). 30 mg. of protein was easily fractionated on a 2.4 × 50 cm. column. The curve representing the distribution of protein in the eluate was characterized by a double-peak containing approximately 75 per cent of the whole material and a second peak which was separated from the first double-peak by one or two fractions with zero protein concentration. By means of starch gel electrophoresis it could be demonstrated that the first double-peak contained H chains and the second peak the L chains. The first part of the H double-peak, however, contained a component of apparently higher molecular weight than was the molecular weight of the H chain. This component is thought to represent an incompletely dissociated antitoxin.

Prior to the determination of protein reacting with the immunosorbent, the fractions or their mixtures were neutralized and diluted with 4 volumes of 0.07 M sodium chloride. The presence of some antibody active material was demonstrated in first fractions of the H peak, i.e. in fractions also containing the incompletely dissociated component. The total amount of protein reacting specifically with the diphtheria immunosorbent in individual fractions is by one order less than the amount which reacts in the sample left without separation under the same conditions. The amount of protein reacting with the immunosorbent can be recorvered to the original level, however, when the material from the peak of the H and L chains is combined. The same effect is obtained when material from the second half of the H double-peak (H-2) is taken for the combination. Since the H-2 peak does not contain any incompletely dissociated component, evidence is given that the recovery effect is due to the apparently inactive H chains. It appears that for such combination no covalent disulphide bond is needed.

In order to investigate whether both chains taking part in the formation of the active site are carriers of antibody specificity, molecular hybrids were prepared from isolated chains of the diphtheria and tetanus antitoxins. The results given in Table 1 indicate that after mixing H and L chains from antibodies of different specificities, a certain amount of active protein is recovered. In these hybrids the active site possesses the specificity of the corresponding H chain. The amount of recovered protein in the hybrid is lower than the amount recovered by combining the chains of a single anti-

Table 1. Recovery of antibody active complexes from different chains of the diphtheria and tetanus antitoxins

Type and origin of chains*	Total amount of protein in the mixture	Amount of protein bound by the immunosorbent with	
		diphtheria anatoxin	tetanus antitoxin
	μg.	μg.	μg.
H_D	3420	7	3
L_D	1860	1	2
H_T	2800	1	8
L_T	1560	0	2
$H_D + L_D$	5280	185	6
$H_T + L_T$	4360	1	25
$H_D + L_T$	4980	35	7
$H_T + L_D$	4660	5	19

*H_D and H_T — H chains from diphtheria and tetanus antitoxins; L_D and L_T — L chains from diphtheria and tetanus antitoxins.

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body. Thus we were able to demonstrate that the antibody specifiity of horse antitoxins is determined by the H chain, but that the presence of L chain is necessary for the formation of a fully active combining site. The L chains of one antibody cannot be fully substituted by L chains from an antibody of another specificity isolated from another individual. It is necessary to note that we found differences in the behaviour of diphtheria antitoxins from different individuals. The ratio of the residual activity in incompletely dissociated antibody and of the recovered activity was in some cases less than one order.

More precise results are expected to be found with antibodies to a single antigenic determinant, e.g. antihapten antibodies.

The authors wish to thank Dr. J. Šterzl for his continued interest in this study. We are indebted to Ing. F. Škvařil from the Institute for Production of Vaccines and Sera, Prague, for providing immune plasmas and to Pharmacia, Uppsala for the gift of Sephadex G-100. We are also thankful to Mr. J. Stránský for skilled technical assistance.

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ВОЗОБНОВЛЕНИЕ АКТИВНОСТИ СВЯЗЫВАНИЯ АНТИТЕЛ В РЕЗУЛЬТАТЕ ВЗАИМОДЕЙСТВИЯ РАЗЛИЧНЫХ ПЕПТИДНЫХ ЦЕПЕЙ, ВЫДЕЛЕННЫХ ИЗ ОЧИЩЕННЫХ ЛОШАДИНЫХ АНТИТОКСИНОВ

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Изучалась способность различных пептидных цепей в лошадиных антитоксинах специфически взаимодействовать с анатоксинами, адсорбированными на целлюлозу (иммуносорбенты). Для этого в очищенных антитоксинах разрушали S-сульфонированием реактивные дисульфидные связи и освобождающиеся пептидные цепи отделяли при помощи Sephadex G-100 в 0,05 М муравьиной кислоте с 6 М мочевиной. Только в первых фракциях тяжелых (H) цепей, в которых присутствовали, однако, небольшие количества неполностью расщепленных антител, встречались в небольших количествах белки, специфически адсорбирующиеся на иммуносорбент. Легкие (L) цепи вообще не реагировали с иммуносорбентом. Однако удавалось возобновить активность антитоксинов, смешивая цепи H и L. Такое же действие, хотя количественно менее выраженное, наблюдалось при смешивании цепей H и L из различных антител. Эти молекулярные гибриды обладали специфичностью соответствующих H цепей.