USE OF 2-[(4-AMINOPHENYL)-SULFONYL]-ETHYL HYDROGEN SULFATE FOR THE PREPARATION OF A DEXTRAN-SPECIFIC IMMUNOGEN

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Received 4 August 1971

1. Introduction

About 30 years ago the "Farbwerke Hoechst" Company, Germany, started developing a series of reactive dyes and reagents [1–3] which react with hydroxyl groups of cellulose (cotton) as follows:

$$R{-}SO_2{-}CH_2{-}CH_2{-}OSO_3H \xrightarrow[\text{cellulose}]{OH^-} R{-}SO_2{-}CH_2{-}CH_2{-}O{-}\text{cellulose}$$

One of the reagents in which R = 4-aminophenyl was designed to allow azo coupling to phenol groups subsequent to the etherization step. It occurred to us that this reagent (p-$H_2N{-}C_6H_4{-}SO_2{-}CH_2{-}CH_2{-}OSO_3H$, 2-[(4-aminophenyl)-sulfonyl]-ethyl hydrogen sulfate) might enable the coupling of polysaccharides to protein carriers and thus be useful for the preparation of artificial immunogens with polysaccharide specificity.

The procedure hitherto most commonly used for such couplings was devised by Goebel and Avery [4] in 1931 and essentially consists of preparation of 4-nitrobenzyl ethers of the polysaccharide followed by reduction and azo coupling. Only very recently two alternative and more convenient methods became available, in which either cyanogen bromide [5] or cyanuric chloride [6] is used as the coupling reagent. The present paper describes the application of 2-[(4-aminophenyl)-sulfonyl]-ethyl hydrogen sulfate (APSE hydrogen sulfate) for preparing a dextran–ethyl–sulfonyl–phenyl–azo-edestin conjugate, which was subsequently used for producing anti-dextran-antibodies in rabbits.

Abbreviations:
APSE: 2-[(4-aminophenyl)-sulfonyl]-ethyl
FCA : Freund's complete adjuvant

2. Experimental

2.1. *Materials*

APSE hydrogen sulfate was a donation of Farbwerke Hoechst, Hoechst, Germany. Edestin was purchased from Serva, Heidelberg, Germany, and dextran (average M.W. = 40,000) was a gift from Knoll, Ludwigshafen/Rh., Germany.

2.2. *Preparation of APSE-ethers of dextran*

Procedure (A) Dextran (100 mg) and APSE hydrogen sulfate (100 mg) were dissolved in 100 ml of buffer (cf. table 1) and kept at room temperature with magnetic stirring for 1 hr. The mixture was neutralized and dialyzed against water. The inner dialysate was then chromatographed on Sephadex G-25, using water as eluent. The yields of conjugate averaged 90 mg. The degrees of substitution as listed in table 1 were determined by measuring the UV-absorption at 265 nm ($\epsilon_{265\ nm}$ = 16,600; solvent: 0.1 N NaOH).

Procedure (B) Dextran (1.5 g), APSE hydrogen sulfate (1.5 g), and barium oxide (3.5 g) in 150 ml of water were continuously shaken for 4 hr at 50°. After centrifugation the supernatant was neutral-

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Table 1
Effect of buffer pH on degree of substitution in preparation of APSE-ethers of dextran at 20° for 1 hr.

pH of buffer (0.5 N HCO_3^-/CO_3^{2-}; NaOH)	Degree of substitution (APSE-groups/monosaccharide unit)
11.0	0.0006
12.0	0.0011
12.5	0.038
13.0	0.078

ized and then treated as described under 2.1 (A). The yield was 1.24 g and the degree of substitution 0.044.

2.3. *Coupling to edestin*

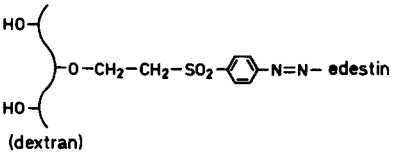
Dextran–APSE (50 mg prepared according to 2.2 (B)) was dissolved in 4 ml of 0.025 N HCl and, at 0°, diazotized by addition of $NaNO_2$ (1.4 mg in 0.7 ml of H_2O). After 20 min (0°) the mixture was added to a solution of edestin (80 mg) in 15 ml of 0.2 N NaOH and allowed to react at 0° for 2 hr. The mixture was concentrated by ultrafiltration and the product was purified by chromatography on Sephadex G-75. The yield was 75 mg of conjugate containing 55 mg of edestin and 20 mg of dextran (protein estimated by the Folin [7] and sugar by the anthrone [8] method).

2.4. *Immunization of rabbits*

15 white New Zealand rabbits were given primary injections of 500 μg (per animal) of the above antigen (with FCA, 2/3 intramuscularly, 1/3 subcutaneously) on day 0. A second injection of 1.0 mg of antigen per animal was made on day 26 (with FCA, intraperitoneally). Test bleedings were taken on day 35. The titres of anti-dextran-antibodies were estimated in precipitin tests carried out according to the common procedure [9] with dextran as test antigen. For hemagglutinin tests sheep erythrocytes were sensitized with dextran stearate as described by Hämmerling and Westphal [10].

3. Results and discussion

APSE hydrogen sulfate was used as coupling reagent for the preparation of a dextran specific immunogen whose structure is outlined below.



The conjugation of dextran to edestin was effected in two steps: (1) reaction of APSE hydrogen sulfate with dextran in alkaline solution, (2) azo coupling to edestin.

For (1): A sufficiently high pH is needed for successful etherization, as can be seen from table 1. If the polysaccharide withstands treatment at a pH of about 13, solid barium oxide may be used for buffering the reaction mixture (procedure 2.2 (B)). Under the conditions employed, this leads to degrees of substitution in the order of 0.05 (one substituent at every twentieth monosaccharide unit).

For (2): Edestin had been shown to possess good carrier properties for carbohydrate haptens in earlier immunization experiments by Hämmerling [11].

Upon injection of a dextran-edestin conjugate prepared according to sect. 2.3 (dextran 26.7% of the total weight of the product) into rabbits all animals responded by producing anti-dextran-antibodies. The precipitin titres of the final sera amounted to 1.3 ± 0.5 mg dextran specific antibody/ml of serum, whereas passive hemagglutinin titres (cf. sect. 2.4) varied between $1:2^{10}$ and $1:2^{12}$ (for comparison: titres of preimmunization sera: $\leqslant 1:2^3$).

The above results show that dextran can be conveniently rendered immunogenic for rabbits by preparing dextran–ethyl–sulfonyl–phenyl–azo-edestin conjugates. The method appears to be more generally applicable as long as the polysaccharides withstand alkaline treatment at pH's of 12–13 for short periods at about 20°.

Acknowledgement

We wish to thank Farbwerke Hoechst (Hoechst, Germany) for donating the reagent 2-[(4-amino-phenyl)-sulfonyl]-ethyl hydrogen sulfate.

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