A CONJUGATE OF PHALLACIDIN WITH BOVINE SERUM ALBUMIN

Th. WIELAND and A. BUKU
Max Planck Institut für Medizinische Forschung, Abteilung Chemie, Heidelberg, Germany

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1. Introduction

Several years ago [1] attempts have been made to obtain an antigenic conjugate of the mushroom toxin β-amanitin [2] with bovine serum albumin (BSA) by using the thiophenylester method of amide coupling [3]. The conjugate, however, proved to be highly toxic even after prolonged dialysis thus being not practicable for antibody production by rabbits. Cessi and Fiume [4], using the carbodiimide method [5], prepared a conjugate of β-amanitin with BSA (molar ratio 2.3:1) whose toxicity was quantitatively evaluated in white mice. On a molar basis the conjugated toxin turned out to be about 10 times more toxic than the low molecular compound (minimum LD 0.8 mg/kg body wt).

In order to learn if there is a similar situation also with the other class of Amanita poisons – the phallotoxins [2] – the carboxylic group of phallacidin (PHC) [6] was connected with BSA.

2. Methods

A solution of 20 mg PHC (23 μM), 7.12 mg 1-ethyl-3-(dimethylamino-propyl)-carbodiimide (46 μM) and 40 mg BSA (0.57 μM) in 2 ml of water was stored for 24 hr at 20°. After adding 2 ml of a 0.1% buffer solution of NH_4HCO_3 it was poured on a column of 2.5 × 130 cm of Sephadex G 75 which has been equilibrated with a 0.05% NH_4HCO_3 buffer, chromatographed using the same buffer, and collected in 10 ml portions under control with a Uvicord photometer. Fractions nos. 15–21 contained the PHC-BSA conjugate, fractions Nos. 22–28 the unreacted albumin, and Nos. 50–70 exceeding PHC. After 24 hr dialysing against water and freeze drying 29 mg of the conjugate were obtained.

3. Results

The existence of PHC covalently bound to the protein was confirmed qualitatively by thin layer chromatography of the DANS-derivatives after total hydrolysis with 6 N HCl. Here the derivative of γ,δ-dihydroxyleucine lactone, a constituent of PHC [6] was detected and identified*.

The PHC content of the conjugate was determined quantitatively by spectrophotometry at 300 nm. At that wave length the extinction of RSA is relatively low as compared with PHC: E_{300} of a 5×10^{-5} M aqueous solution of RSA = 0.14, of PHC-RSA = 1.70, of PHC = 0.57. The difference of 1.56 corresponded to 2.74 moles PHC bound per mole RSA.

The conjugate showed no toxicity at the white mouse up to 200 mg per kg (corresponding to circa 6 mg PHC). Since LD_{50} of PHC = 2.5 mg/kg, this means that binding PHC to RSA results in a detoxification of the phytotoxin.

References

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* The identification of all of the aminolactones of the amanitatoxins by the DANS technique will be published elsewhere.

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