GLUTATHIONE-BOUND CELLULOSES: PREPARATION WITH THE LINKING REAGENT S-TRIAZINE TRICHLORIDE AND USE IN CHROMATOGRAPHY

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Glutathione has been covalently bound to cellulose via the linking reagent s-triazine trichloride (sTT) in three ways: the tripeptide through its sulfur and either reduced or oxidized glutathione through its amino nitrogen. The use of glutathione-bound cellulose in chromatography was studied with bovine serum albumin (BSA) and glutathione reductase. The enzyme was eluted with 1 M NaCl.

INTRODUCTION

A number of methods have been described in which thiols or disulfides including glutathione were covalently bound to solid supports (1–8). Our interest in glutathione-requiring proteins led us to search for a system in which glutathione could be bound through either its sulfur or amino nitrogen. In addition, a nonporous carrier was desired in order to alleviate any problems that might arise due to entrapment of substances within the gel pores. Cellulose activated by s-triazine trichloride (sTT) has been described and used for binding amino acids and proteins (9). This procedure has been utilized in preparing three cellulose derivatives of bound glutathione: one in which oxidized glutathione is bound through the amino group, and two others in which the tripeptide is bound through either the amino or thiol group.

MATERIALS

Chemicals were purchased from the following companies: s-triazine trichloride, Aldrich Chemical Company, Milwaukee, Wisconsin; reduced glutathione, oxidized glutathione, nicotinamide adenine dinucleotide phosphate (reduced form) and bovine serum albumin (BSA), Sigma.
Chemical Company, St. Louis, Missouri; glutathione reductase, Boehringer–Mannheim Biochemicals, New York; dioxane and naphthalene (scintillation grade), Matheson, Coleman and Bell, Los Angeles, California; and 2,5-diphenyloxazole (PPO), Beckman Industries, Fullerton, California. $^3$H-glutathione (250 mCi/mmol) was purchased from New England Nuclear Corporation, Boston, Massachusetts. The following celluloses were used: cellophane dialysis tubing (1½ in.), H. A. Thomas, Philadelphia, Pennsylvania; cotton, Parke Davis, Los Angeles; and fibrous cellulose (column Chromedia-Grade CF11), Whatman–Reeve Angel, Clifton, New Jersey. All other reagents were reagent grade.

METHODS

Activation of cellulose by sTT was carried out according to the procedure of Smith and Lenhoff (9). In the case of cellophane, the dialysis tubing was cut open and flattened between two pieces of Teflon mesh to provide for maximum exposure to the activating solution. Cotton and fibrous cellulose were transferred to the various solutions after filtration on coarse-grade sintered glass. Precautions were taken with fibrous cellulose particles because they tended to clump in the dioxane–xylene solvent; this difficulty was overcome by using 105–210 μm particles. The activated species will be referred to as sTT-cellophane, sTT-cotton, or sTT-cellulose. The various activated cellulosic species were either used immediately or stored in acetone for no more than 36 h prior to use.

Two control celluloses were prepared. Control A, used for experiments with $^3$H-GSH, consisted of a cellulosic species that had been taken through the activation process in the absence of sTT. In the case of cellophane, control A was turbid and brittle as opposed to the transparent, flexible quality exhibited by sTT-cellophane.

Control B was sTT-cellulose that was allowed to remain in 0.1 M phosphate, pH 7.7, for 5–20 h, and then washed as described below. In the resultant triazine-bound cellulose, all reactive chlorines were replaced by hydroxyl groups (9,10). In an experiment designed to measure the binding of $^3$H-GSH to sTT-cellulose, approximately 2% of the counts could be accounted for by reacting with this control. Control B contributed slightly to the ninhydrin assay, probably a reflection of the lability of the triazine nucleus when hydroxyl groups are present (10). Control B cellulose was also used in chromatographic studies to assess the effect of the triazine nucleus on the protein under study.

Prior to reacting with glutathione, the activated cellulosic species was hydrated with cold buffer. Attachment was carried out at room temperature.