ABSTRACT  SI proteins (A, B, C and D) are a group of nuclear proteins, isolated by lowering pH to 4.9 of the reaction supernatant of hepatocyte nuclei that had been mildly digested with DNase I. Protein B, apparently ubiquitous in vertebrate cells, was prepared from rat liver and used to immunize a rabbit. The raised antiserum specifically reacted with SI proteins; it reacted not only with protein B, but also with C and D. Immunoblotting demonstrated that these proteins occurred exclusively in the nucleus, being absent in the cytosol, microsome and mitochondrial fractions. Indirect immunofluorescence of liver tissue sections confirmed their nuclear localization, and further showed that the antibody selectively stained extranucleolar regions of the cell nucleus. These findings suggest that the anti-Si antibody is specific to SI proteins and may be useful for their structural and functional studies.

MATERIALS AND METHODS

Chemicals. Chemicals were purchased from Sigma, except for those specified below.

SI protein preparation. SI proteins were prepared from isolated nuclei of livers of various vertebrates (rat, dog, chicken, frog and carp) and of calf thymus, according to the method described previously (1). Briefly, liver nuclei, isolated through 9 tissue volumes (v/w) of 2.3 M sucrose/3 mM MgCl₂/0.2 mM phenylmethylsulfonyl fluoride, were washed extensively with 0.3 M sucrose/3 mM MgCl₂/0.2 mM phenylmethylsulfonyl fluoride/12 mM Tris-HCl, pH 7.6 (SMT), and digested at 30 A₂₆₀ units/ml with DNase I (2.0-4.5 µg/ml, Sigma) at 30°C for 7 min in 0.5 mM CaCl₂/SMT. The reaction was terminated with Na₂EDTA (5 mM), the preparation centrifuged at 50,000 x g, and the supernatant was concentrated by precipitation with 2 volumes cold ethanol (in the presence of 0.1 M NaCl). In SDS-PAGE, they are further split into closely spaced doublets (A₁ and A₂, B₁ and B₂, C₁ and C₂), and a triplet (D₁, D₂ and D₃). Based on their copy numbers, which are greater than those of the high mobility group proteins, SI proteins are thought to be structural constituents of the cell nucleus; however, they do not coincide with the known nuclear structural proteins (1). In this study, we raised in a rabbit an antiserum against protein B from rat liver, and examined its specificity and the intracellular localization of SI proteins.
SDS.

Protein concentration was determined using bovine serum albumin as a standard, according to the manufacturer’s protocol (Pierce, BCA protein assay reagent). Electrophoresis was carried out as described by Laemmli (4).

**Immunization.** SI proteins (1.5 mg) were separated by electrophoresis on a 11% polyacrylamide SDS-slab gel (2 x 150 x 150 mm) with 3% stacking gel (10 mm high). After electrophoresis, the gel was stained with Coomassie blue for 2 min and destained. Using a razor blade, band B was excised, and dialyzed against 10 mM phosphate buffer, pH 7.5. Two female New Zealand white rabbits (4 kg) were immunized subcutaneously with 60-100 µg protein, in the gel pieces that were crushed finely and emulsified with Freund’s complete adjuvant. Animals were immunized three times at 2 week intervals using incomplete adjuvant and bled 7 days after the last booster injection. Sera were divided into aliquots and stored at -20°C.

Before immunization, sera were collected from the ear veins as preimmune controls.

**Immunoblotting.** Western blotting of SI proteins was performed according to the method of Towbin et al. (5). The blotted-polyvinylidene difluoride (PVDF) membrane (Millipore) was blocked with 2% non-fat milk in 10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 8.0 (TBST) for 30 min and incubated with anti-SI antibody (1:300 dilution in TBST) for 60 min. After washing 3 times with TBST, the membrane was further incubated with peroxidase-conjugated goat anti-rabbit IgG (ab')2 (1:1500, Cappel Lab., USA) for 60 min, washed and finally incubated with 0.015% H2O2 and 0.4 mg/ml 3-amino-9-ethylcarbazole in 50 mM sodium acetate, pH 4.9, until the color developed.

**RESULTS**

Common occurrence of protein B (Fig. 1). SDS-gel electrophoresis demonstrated the presence of proteins A and B in SI protein preparations from hepatic or thymic cells of various vertebrates. Although protein C seemed to be present in the samples, the stained inten-

![Fig. 1. SDS-PAGE of SI proteins from various vertebrates.](image)

SI proteins were prepared from the nuclei isolated from livers of rat (lane 1), dog (lane 2), chicken (lane 4), frog (lane 5), and carp (lane 6), and from calf thymus (lane 3), and electrophoresed on SDS gels. The gels were stained with Coomassie blue. Mr. was determined using bovine serum albumin (68,000), chicken egg albumin (43,000) and bovine pancreatic DNase I (30,000) as standards.

![Fig. 2. Immunoblotting.](image)

**Immunofluorescence.** Rat liver was excised, quickly frozen in acetone-dry ice, and 10 µm thick sections were cut by a Bright cryostat at -15°C. The sections on slide glasses were dried in air for 15 min, fixed with 10% formaldehyde in phosphate buffered saline (PBS) for 10 min, and permeabilized in 0.1% Triton X-100/PBS for 5 min. After three washings in PBS, they were overlaid with anti-SI antibody diluted at 1:50 for 15 h at 4°C, and washed three times. They were successively incubated for 20 min with FITC-conjugated goat anti-rabbit IgG (Cappel Lab., USA) diluted at 1:20, washed, mounted in a GEL mount (Biomeda Corp., USA) and observed by an immunofluorescence microscope (Olympus, Japan).
Antibody to SI Proteins

Fig. 3. SI proteins are exclusively present in the cell nucleus. Proteins (75 μg) of the mitochondria (lane 1), microsome (lane 3) and cytosol (lane 4) fractions and SI proteins (6 μg, lane 2) from rat liver were resolved by SDS-PAGE, and blotted on PVDF membrane. The membrane was first stained with Coomassie blue (lanes 1-4); then destained in 50% methanol and in 90% acetonitrile, washed and reacted with antibodies (lane 1'-4'). Note that no immunopositive bands were detected in the post nuclear fractions (lanes 1', 3' and 4').

Fig. 4. Fluorescence micrographs of rat liver. Rat liver cryostat sections were stained with anti-SI antibody (A) or with preimmune rabbit serum (B), and then with FITC-conjugated anti-rabbit IgG. Note the strong fluorescence in the extranucleolar nuclear region in (A). Magnification ×200.

DISCUSSION

Proteins A and B were commonly found in the cells of various vertebrates: mammals, bird, amphibian and fish. It is likely that these proteins are ubiquitous in
higher eukaryotic cells.

The anti-S1 antibody, raised against protein B, not only reacted with protein B, but also with C and D. The result suggests that proteins B, C and D had common epitopes (our recent study on monoclonal antibodies against S1 proteins supports the presence of common epitope(s) in these proteins (unpublished)). The antibody did not, however, react with all S1 proteins: only proteins B2, C1 and D1 were stained. Their epitope structure(s) is currently unknown. Possibly, a postsynthetic modification could give rise to the epitope(s).

Both immunoblotting and immunostaining demonstrated that S1 proteins were present only in the cell nucleus. The fluorescence study further showed that nucleoli were not stained with anti-S1 antibody: the S1 proteins may not be involved in the production of ribosomes. The function of S1 proteins is, however, unknown. S1 proteins are released with DNase I from isolated nuclei (1) under the conditions which rapidly fragment the transcriptionally active chromatin (7, 8). S1 proteins are also released from nuclei by digestion with RNase (3). These may suggest that S1 proteins exist in association with RNA-containing chromatin structures and participate in the transcriptional (or posttranscriptional) process. The anti-S1 antibody will become a useful tool for understanding the function of S1 proteins.

In conclusion, the present study suggests that S1 proteins are located in the cell nucleus, and that the proteins B, C and D have common epitopes. The anti-S1 antibody may be used as a specific probe in structural and functional studies of S1 proteins.

Acknowledgment. We thank Dr. Naoki Yamamoto for his critical reading of the manuscript.

REFERENCES


(Received for publication, January 18, 1990 and in revised form, March 8, 1990)