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Biochemistry

A multisubunit complex containing the *SWI1/ADR6, SWI2/SNF2, SWI3, SNF5,* and *SNF6* gene products isolated from yeast

(transcription/DNA-dependent ATPase/mating type switching)

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ABSTRACT **A complex containing the products of the *SWI1/ADR6, SWI2/SNF2, SWI3, SNF5,* and *SNF6* genes and four additional polypeptides has been purified from extracts of the yeast *Saccharomyces cerevisiae*. Physical association of these proteins was demonstrated by copurification and coimmunoprecipitation. A potent DNA-dependent ATPase copurified with the complex, and this activity was evidently associated with SWI2/SNF2.**

Genetic studies have shown that the products of the *SWI1/ADR6, SWI2/SNF2, SWI3, SNF5,* and *SNF6* genes play related roles in the transcriptional regulation of several yeast genes (1). All five proteins are required for proper control of the *HO* gene (which encodes an endonuclease required for mating type switching) (2–6), the *SUC2* gene (which encodes invertase) (7–10), the *GAL1* gene (which encodes galactokinase) (7), the *ADH2* gene (which encodes alcohol dehydrogenase) (7, 11, 12), and Ty elements (13, 14). These SWI and SNF proteins do not appear to contain any DNA-binding motifs, nor have these proteins exhibited any DNA-binding activity *in vitro* (7, 9, 15). SWI2/SNF2, however, contains a domain homologous to certain DNA-dependent ATPases, and a recombinant form of this domain exhibits DNA-dependent ATPase activity (16–18). Genetic data suggest that these SWI and SNF proteins form a complex that is recruited to promoter regions through interactions with gene-specific DNA-binding proteins (7, 9, 19). In addition, physical association of SWI3 with a transcriptional activator has been demonstrated (20). Here we present biochemical evidence that these five proteins are indeed associated in a complex and further demonstrate that this complex contains at least four additional proteins (termed SWPs, for SWI/SNF-associated proteins).

MATERIALS AND METHODS

Purification of the SWP Complex. Preparation of yeast (*Saccharomyces cerevisiae*) whole cell extracts (from BJ926) and the first three chromatographic steps (Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite) were performed as described for the purification of yeast initiation fraction f (21). The peak of the SWP complex eluted with fraction f activity from Bio-Rex 70 and DEAE-Sephacel but following fraction f activity from hydroxylapatite. Additional experiments have demonstrated that fraction f is not the SWP complex (unpublished observations). The peak of the SWP complex eluted from hydroxylapatite at 100 mM phosphate, and peak fractions were further resolved on SP-5-PW and TSK-heparin. Adsorbed proteins were eluted with a linear gradient of 100–600 mM potassium acetate in buffer A (20 mM Hepes-KOH, pH 7.6/20% glycerol/1 mM dithiothreitol/1 mM EDTA/2 μg of chymostatin per ml/2 μM pepstatin A/0.6 μM leupeptin/2 mM benzamidine/1 mM phenylmethylsulfonyl fluoride/0.01% Nonidet P-40). The purification was monitored by immunoblot analysis with antisera to SWI1/ADR6, SWI2/SNF2, and SWI3 proteins.

Preparation of Recombinant SNF6 Protein. The SNF6 gene was transferred from the plasmid YCp50-SNF6 to the *Escherichia coli* expression vector pET11A (Novagen) and tagged with six histidine residues at its carboxyl terminus by means of a polymerase chain reaction with the oligonucleotide primers BCSNF6N (5′-CCCCAGATCTTACATATGGGT-GTCATCAAGAAGAAA-3′) and BCSNF6C (5′-CCCG-GATCCTCTAGACTAGTGATGGTGATGGTGATGTC-CAAAAAATACAGCATCAAGATCTCC-3′). The resulting product was digested with *Nde* I and *Bam*HI and ligated to complementary sites in pET11A to form pET11A-SNF6HT. BL21 (DE3) cells (Novagen) containing the lysozyme-expressing plasmid pLysS (Novagen) were transformed with pET11A-SNF6HT. The transformant was grown at 30°C in LB medium (2 liters) containing 100 μg of ampicillin per ml and 25 μg of chloramphenicol per ml to an optical density at 600 nm of 0.7. The culture was induced with 0.5 mM isopropyl β-D-thiogalactoside and grown an additional 2 hr. The cells were harvested by centrifugation at 5000 × *g* for 10 min, washed with 100 ml of 20 mM Hepes (pH 7.5), and suspended in 100 ml of buffer B (20 mM Hepes, pH 7.5/10% glycerol/5 mM 2-mercaptoethanol/1 mM EDTA/2 μg of chymostatin per ml/2 μM pepstatin A/0.6 μM leupeptin/2 mM benzamidine/1 mM phenylmethylsulfonyl fluoride), containing 400 mM NaCl. The suspension was lysed by sonication at 4°C and centrifuged at 10,000 × *g* for 10 min. The pellet, containing the majority of SNF6HT protein, was suspended in 10 ml of buffer-B containing 0.5% Triton X-100 and 1 M lithum chloride, incubated at 4°C for 10 min, sonicated briefly, and centrifuged at 10,000 × *g* for 10 min at 4°C. The pellet was washed three additional times in the same manner and suspended in 1 ml of buffer B containing 50 mM NaCl and 0.5% SDS. The resulting SNF6HT protein was judged to be roughly 95% pure by SDS/PAGE.

Antisera. Approximately 4 mg of purified SNF6HT protein was subjected to electrophoresis in a 10% SDS/polyacrylamide gel. The gel was stained with Coomassie brilliant blue R-250, and a slice containing SNF6HT protein was removed. Rabbits were initially immunized with 0.5 mg of SNF6HT and then given booster injections after 21 and 42 days with 0.25 mg of SNF6HT protein. The SNF6 antiserum used in this study was taken 10 days after the second booster injection and was prepared by BabCO (Berkeley, CA). Antisera were also raised to SWI2/SNF2 and SNF5 against purified recombinant proteins. Antisera to SWI3 and SWI1/ADR6 proteins were gen-

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Abbreviations: IP, immunoprecipitation; SWP, SWI/SNF-associated protein.

erously provided by C. Peterson and E. Young, respectively. An additional antibody to SNF2, used for the initial monitoring of SNF2 fractionation, was generously provided by M. Carlson. All antisera used in this study were obtained from rabbits.

Immunoblot Analysis. All immunoblots (22) were incubated with a 1:500 dilution of primary antibody in Tris-buffered saline/2% milk for 8 hr, followed by a 1-hr incubation in a 1:2000 dilution of goat anti-rabbit secondary antibody (Bio-Rad) conjugated to alkaline phosphatase.

Immunoprecipitation (IP) and Immunodepletion Experiments. To prepare SWI3 immune complexes, affinity-purified SWI3 antibody (4 μl) was equilibrated at 4°C for 30 min with 20 μl of 50% protein A-Sepharose beads in IP buffer (20 mM Hepes, pH 7.6/10% glycerol/12.5 mM $MgCl_2$/0.1 mM EDTA/0.2% Nonidet P-40/0.1 mM dithiothreitol) with 0.1 M potassium acetate. An equal volume of protein A-Sepharose beads was also used to preclear 100 μl (100 μg) of the peak hydroxylapatite fraction. The samples were centrifuged at 4000 rpm for 2 min. The adsorbed SWI3 antibody/bead complex was incubated with the precleared supernatant at 4°C for 3 hr and sedimented at 13,000 rpm in a microcentrifuge for 5 min. The pellet was washed three times with 0.5 ml of IP buffer with 0.1 M potassium acetate and eluted by boiling for 2 min in 40 μl of 2× SDS/PAGE loading buffer. To effect complete immunodepletion of the hydroxylapatite fraction, the supernatant generated from this initial IP (120 μl) was subjected to a second IP with crude SWI3 antiserum coupled to protein A-Sepharose beads (see below), employing the same conditions as for the initial IP.

To prepare anti-SNF6 immune complexes, the peak hydroxylapatite (100 μl; 100 μg) fraction was precleared and immunoprecipitated with 20 μl of 50% anti-SNF6 antibody-protein A-Sepharose beads in IP buffer containing 0.1 M potassium acetate and treated as described above, except the washes were performed with IP buffer containing 0.6 M potassium acetate, and the final wash was performed with IP buffer lacking Hepes. Eight proteins could be eluted from the anti-SNF6 immune complex with two 20-μl elutions with buffer C (50 mM glycine, pH 2.3/150 mM sodium chloride). For immunodepletion experiments involving SNF6 antiserum, the supernatant from the first IP was subjected to a second IP as described above for SWI3 immunodepletion. Immunodepletion of SNF6, SWI3, and SNF5 was verified by protein immunoblotting.

Crude antisera coupled to protein A-Sepharose for second IPs were prepared as follows. Crude antiserum (400 μl) was incubated with 200 μl of 50% protein A-Sepharose beads in phosphate-buffered saline at room temperature for 1 hr. The beads were sedimented and washed twice with 1 ml of 0.2 M sodium borate (pH 9.0) and resuspended in 1 ml of 0.2 M sodium borate (pH 9.0). Solid dimethylpimelimidate was added to a final concentration of 20 mM. The slurry was incubated 30 min at room temperature, sedimented, and further incubated with 0.2 M ethanolamine (pH 8.0) for 2 hr at room temperature.

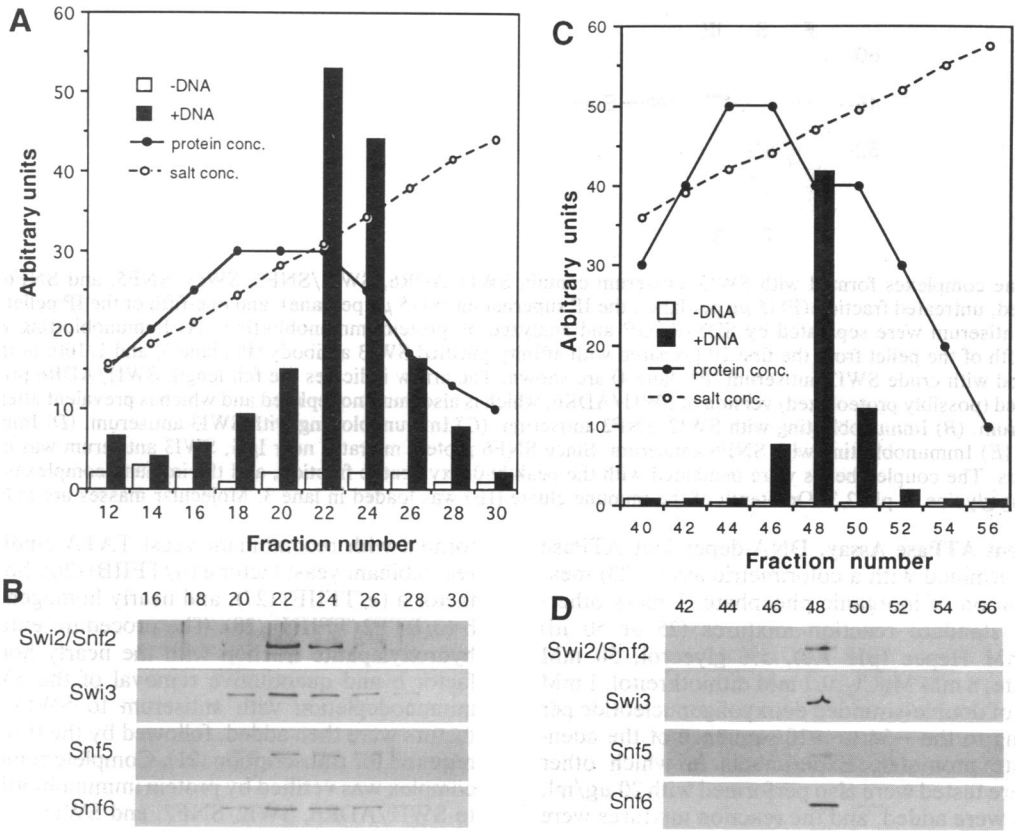

FIG. 1. SWI2/SNF2, SWI3, SNF5, and SNF6 proteins cofractionate with DNA-dependent ATPase activity on SP-5-PW and TSK-heparin. Yeast extracts were fractionated on Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite (21). Peak fractions were further resolved on SP-5-PW and TSK-heparin. ATPase activity was assayed in the absence (open bars) or presence (solid bars) of 20 μg of double-stranded DNA per ml. One arbitrary unit corresponds to the hydrolysis of 1 pmol of ATP per μg of protein per min. ●, Protein concentration in multiples of 10 ng/ml; ○, potassium acetate concentration in multiples of 10 mM. (*A*) SP-5-PW chromatography. Peak fractions from hydroxylapatite were further resolved on SP-5-PW. Adsorbed proteins were eluted in buffer A with a linear gradient of 100–600 mM potassium acetate. (*B*) Fractions from SP-5-PW (2.5 μg per lane) were separated by SDS/PAGE and immunoblotted with polyclonal antisera against SWI2/SNF2, SWI3, SNF5, and SNF6 proteins. Similar results were obtained with SWI1/ADR6 antiserum (not shown). (*C*) TSK-heparin chromatography. Peak fractions from SP-5-PW were further resolved on TSK-heparin. Adsorbed proteins were eluted in buffer A with a linear gradient of 100–600 mM potassium acetate. (*D*) Fractions from SP-5-PW (2.5 μg per lane) were separated by SDS/PAGE and immunoblotted with polyclonal antisera against SWI2/SNF2, SWI3, SNF5, and SNF6 proteins. Similar results were obtained with SWI1/ADR6 antiserum (not shown).

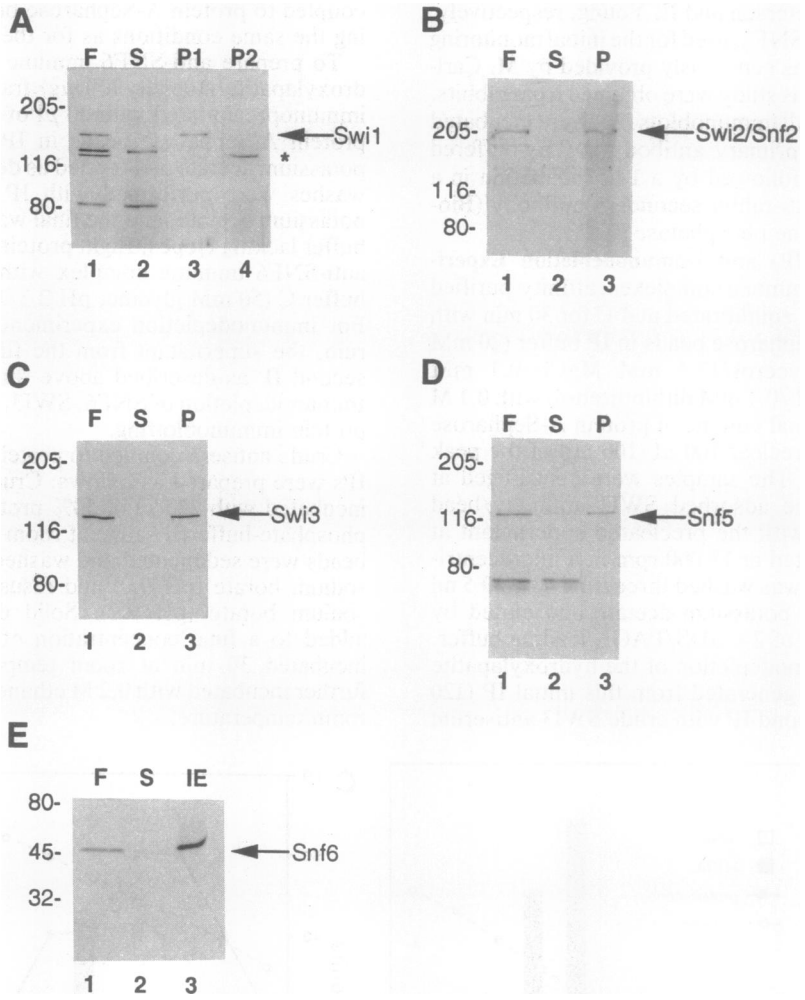

FIG. 2. Immune complexes formed with SWI3 antiserum contain SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 proteins. Unless otherwise indicated, untreated fraction (F) (5 μg per lane), the IP supernatant (S) (5 μg per lane), and one-fifth of the IP pellet (P) acquired with affinity-purified antiserum were separated by SDS/PAGE and analyzed by protein immunoblotting. (*A*) Immunoblotting with SWI1/ADR6 antiserum. One-fifth of the pellet from the first IP prepared with affinity-purified SWI3 antibody (P1, lane 3) and 1/10th of the pellet from the second IP prepared with crude SWI3 antiserum (P2, lane 4) are shown. The arrow indicates the full-length SWI1/ADR6 protein; the asterisk indicates a modified (possibly proteolyzed) version of SWI1/ADR6, which is also immunodepleted and which is prevalent after long incubations with crude antiserum. (*B*) Immunoblotting with SWI2/SNF2 antiserum. (*C*) Immunoblotting with SWI3 antiserum. (*D*) Immunoblotting with SNF5 antiserum. (*E*) Immunoblotting with SNF6 antiserum. Since SNF6 protein migrates near IgG, SWI3 antiserum was coupled to protein A-Sepharose beads. The coupled beads were incubated with the peak hydroxylapatite fraction, and the immune complexes were eluted with a buffer containing glycine at pH 2.3. One-tenth of the immune eluate (IE) was loaded in lane 3. Molecular masses are indicated in kDa.

DNA-Dependent ATPase Assay. DNA-dependent ATPase activity was determined with a colorimetric assay (23) measuring the formation of inorganic phosphate. Unless otherwise indicated, standard reaction mixtures (25 or 50 μl) contained 20 mM Hepes (pH 7.0), 5% glycerol, 50 mM potassium acetate, 8 mM $MgCl_2$, 0.1 mM dithiothreitol, 1 mM ATP, and 20 μg of double-stranded deoxyoligonucleotide per ml corresponding to the −34 to +10 sequence of the adenoviral major late promoter. Experiments in which other nucleic acids were tested were also performed with 20 μg/ml. Protein samples were added, and the reaction mixtures were incubated at 30°C for 30 min. Reactions were terminated by the addition of 0.85 ml of malachite green/acid molybdate solution, followed (1 min later) by the addition of 0.1 ml of a 34% (wt/vol) sodium citrate solution. After 15 min of color development, A_{630} was determined.

Transcription Reactions. The peak hydroxylapatite fraction used in the immunodepletion experiments contained, in addition to the SWP complex, sufficient amounts of RNA polymerase II and initiation factor g (24) to support high levels of transcription from the *CYC1* promoter when complemented with other required factors. Such complementation was performed with recombinant yeast TATA-binding protein (25), recombinant yeast factor e (α/TFIIB) (26), homogenous yeast factor a (ε/TFIIE) (27), and nearly homogenous yeast factor b (δ/BTF2/TFIIH) (28). The procedure entailed mixing the hydroxylapatite fraction with the nearly homogenous yeast factor b and quantitative removal of the SWP complex by immunodepletion with antiserum to SWI3. The remaining factors were then added, followed by the further components required for transcription (21). Complete removal of the SWP complex was verified by protein immunoblotting with antisera to SWI1/ADR6, SWI2/SNF2, and SWI3.

RESULTS

Fractionation of yeast extracts was monitored by Western blot analysis (22) with antisera to SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 proteins and by a DNA-dependent ATPase assay. The five proteins cochromatographed on Bio-Rex 70, DEAE-Sephacel, hydroxylapatite, SP-5-PW, and TSK-heparin (Fig. 1 and data not shown). Further evidence for a physical association of these proteins came from immunodepletion experiments with SWI3 antiserum (Fig. 2). Immunodepletion of SWI3 protein from the peak

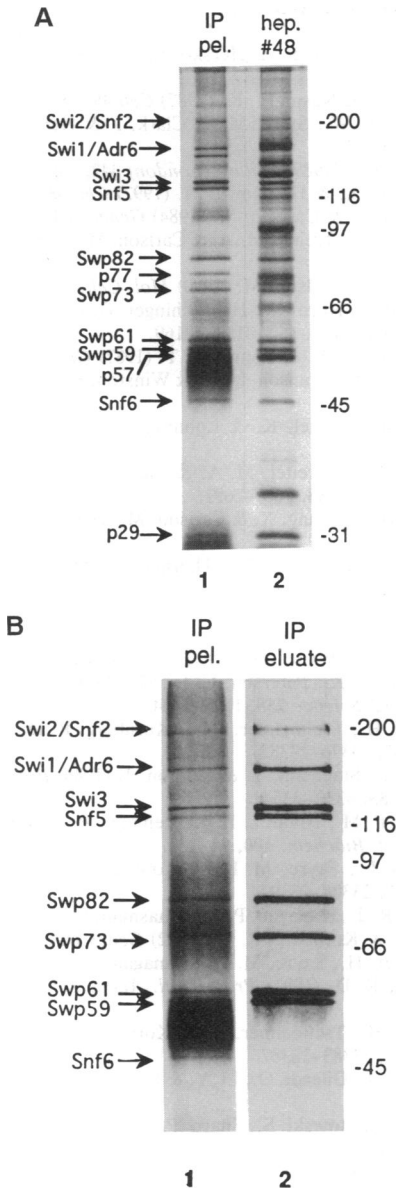

FIG. 3. SDS/PAGE analysis of immune complexes and of a highly purified protein fraction. (*A*) SDS/PAGE analysis of anti-SWI3 immune complexes and the peak TSK-heparin fraction. One-half of the anti-SWI3 immune precipitate (lane 1) and 2 μg of the peak fraction (#48) from TSK-heparin [concentrated by precipitation with 10% trichloroacetic acid and 0.2% (wt/vol) sodium deoxycholate carrier] (lane 2) were separated in an SDS/9% polyacrylamide gel. (*B*) SDS/PAGE analysis of the anti-SNF6 immune complex and immune eluate. One-third of the anti-SNF6 immune precipitate (lane 1) and one-half of the immune eluate with glycine (pH 2.3) (lane 2) were separated in an SDS/9% polyacrylamide gel. Proteins were revealed by staining with silver. Molecular masses are indicated in kDa.

hydroxylapatite fraction resulted in immunodepletion of SWI1/ADR6, SWI2/SNF2, SNF5, and SNF6 proteins as well. Immunodepletion experiments performed with SNF6 antiserum yielded similar results (Fig. 3, and data not shown). In addition, all five proteins could be identified in the SWI3 and SNF6 immune precipitates (Fig. 3). SDS/PAGE of the peak fraction from TSK-heparin and of the SWI3 immune precipitate from hydroxylapatite revealed 12 polypeptides that comigrated in the gel and stained with similar intensity (Fig. 3*A*). The apparent molecular masses of these 12 proteins were 205, 165, 130, 120, 82, 77, 73, 61, 59, 57, 47, and 29 kDa.

Table 1. Nucleic acid specificity of the SWP complex ATPase activity

DNA coeffector	Activity, %
Single-stranded	
M13	94
Reverse primer	16
Forward primer	15
Double-stranded	
BSCR	89
pUC18	91
pSP73	99
AdMLP (oligonucleotide)	100
Other	
tRNA	13
Poly(dI)·poly(dC)	1

Activities elicited by each nucleic acid are reported relative to those observed in the optimal reaction. A nucleic acid concentration of 20 μg/ml was employed in all reactions. BSCR, Bluescript; AdMLP, adenoviral major late promoter.

Western blot analysis demonstrated that SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 proteins migrate with apparent molecular masses of 165, 205, 130, 120, and 47 kDa, respectively (Fig. 2, and data not shown). Three additional polypeptides present in the SWI3 immune precipitate, p77, p57, and p29, could not be shown to cofractionate on TSK-heparin, due to the presence of other comigrating polypeptides in fractions flanking the peak. An additional polypeptide with an apparent molecular mass of 155 kDa present in SWI3 immune precipitates did not stain as intensely in the peak TSK-heparin fraction, indicating that this protein may be loosely associated with the complex.

Analysis of the anti-SNF6 immune precipitate revealed nine polypeptides that migrated with apparent molecular masses of 205, 165, 130, 120, 82, 73, 61, 59, and 47 kDa (Fig. 3*B*). The differences in the polypeptide compositions of the anti-SNF6 and anti-SWI3 immune precipitates were presumably due to the higher salt concentration in the buffer used to wash the anti-SNF6 immune complexes. Immunoblot analysis indicated that the 165-, 205-, 130-, 120-, and 47-kDa proteins were SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 proteins, respectively (data not shown). Eight of these nine proteins could be eluted from the anti-SNF6 immune complex with a pH 2.3 glycine buffer (Fig. 3*B*, lane 2). Under these elution conditions, SNF6 remained bound to the antibody-conjugated beads. Further SDS/PAGE and immunoblot analysis established that TATA-binding protein (29, 30), RNA polymerase, SRB2, SRB4, SRB5, and SRB6 (31) are not components of the SWP complex (data not shown). These results suggest that SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, SNF6, and at least four additional polypeptides, SWP 82, 73, 61, and 59, are associated in a complex.

Recent studies have demonstrated that a recombinant form of SWI2/SNF2 protein exhibits DNA-dependent ATPase activity (18), but the possible activity of SWI2/SNF2 protein isolated from yeast has not been investigated biochemically. DNA-dependent ATPase activity copurified with the SWP complex on SP-5-PW and TSK-heparin (Fig. 1). This activity was removed by immunodepletion of the peak SP-5-PW fraction (100 μl:30 μg) with affinity-purified and crude SWI3 antisera. The supernatant exhibited only 11% of the activity observed with the untreated fraction. Incubation of the peak SP-5-PW fraction with SWI2/SNF2 antiserum inhibited activity by 40%, whereas no inhibition was observed with preimmune serum, indicating that the activity is associated with the SWI2/SNF2 component of the complex.

DNA-dependent ATPase activity required a divalent cation and was optimal at a $MgCl_2$ concentration of 7–10 mM. Substitution of $MgCl_2$ with 10 mM $MnCl_2$, $CaCl_2$, or $ZnCl_2$

resulted in 75%, 28%, or 1% of the optimal activity, respectively. Concentrations of potassium acetate below 150 mM or sodium chloride below 50 mM did not inhibit activity. Raising the temperature from 30°C to 37°C, under optimal conditions, increased the activity by 50%. The activity exhibited a broad pH optimum, with maximal activity observed at pH 6.5–8.0. Various double-stranded DNAs elicited maximal activity (Table 1). Single-stranded M13 DNA also was a potent effector of the activity. The presence of a free 5′ or 3′ end was not required since supercoiled plasmid DNA also supported the activity. In contrast, poly(dI)·poly(dC) was ineffective. Short, single-stranded deoxyoligonucleotides and tRNA could only elicit weak activity. The conditions for optimal activity revealed in this study are consistent with those observed with a recombinant version of the SWI2/SNF2 DNA-dependent ATPase domain (18), although the specific activity observed with yeast SWI2/SNF2 protein is at least 10-fold greater than that observed with the recombinant protein.

Although genetic experiments have implicated the SWI/SNF proteins in regulated transcription, their possible involvement in basal transcription in yeast has not been tested biochemically. To this end, RNA polymerase II transcription of the *CYC1* promoter was performed with a set of purified components (24–28) and the peak hydroxylàpatite fraction described above, which contained RNA polymerase II and the SWP complex. The level of transcription was the same whether or not the hydroxylapatite fraction had been immunodepleted with SWI3 antiserum. Evidently, the SWP complex has no effect on transcription of the *CYC1* promoter *in vitro* under these conditions. Future studies must be directed toward a possible direct role for the SWP complex in activated transcription.

DISCUSSION

Several lines of evidence suggest a connection between SWI and SNF proteins and chromatin structure (1). First, selections for extragenic suppressors of *swi* and *snf* mutants yielded mutations in genes encoding histones and putative non-histone chromatin components (32–34). Two of these suppressors, *sin1/spt2* and *ssn20/spt6*, were also identified during selections for extragenic suppressors of insertion mutations caused by Ty elements (termed *spt* mutants for suppressor of Ty) (35, 36). The phenotypes conferred by mutations in certain *SPT* genes strongly support the involvement of their gene products in the establishment or maintenance of chromatin structure, and suppression analyses suggest that *SWI* and *SNF* gene products antagonize SPT protein functions (37, 38). Furthermore, chromatin structure on the *SUC2* promoter is altered in *snf2* and *snf5* mutants (38). Finally, homologs of *SWI2/SNF2* have been identified in yeast (16, 17), *Drosophila* (39, 40), mouse (41), and humans (42, 43), and the protein encoded by the *Drosophila brm* gene appears to affect the transcription of several genes by assisting in the remodeling of chromatin structure (44). Thus, complexes analogous to the yeast SWP complex may help in general to mediate the transition from the repressed to the derepressed state of chromatin. Cloning of other components of the SWP complex may reveal additional proteins required for associations with activators, chromatin components, or the transcription machinery itself.

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