phenotype was conferred in combination with *gcn5Δ* (data not shown), suggesting that the wing might assist BD2 in H3K14ac recognition.

H3K14ac Binds Preferentially to Rsc4 BD2

We took both in vivo and in vitro approaches to determine which of the two Rsc4 bromodomains binds H3K14ac. Our in vivo approach involved isolating mutations specifically impaired at individual bromodomains and testing them in combination with *gcn5*Δ and *h3*K14 mutations. First, we made mutations in BD1 or BD2 of two tyrosine residues that are highly conserved in all bromodomains (Figure S3) and are important for recognizing the acetyl moiety in acetyl-lysine. Replacement of these tyrosines with alanine resulted in the *rsc4* alleles *rsc4* Y92A Y93A (BD1 mutant), *rsc4* Y225A Y226A (BD2 mutant), and the combined *rsc4* Y92A Y93A Y225A Y226A (BD1&2 mutant) alleles. Each encoded a stable derivative that was fully capable of assembly into the RSC complex (data not shown). The isolated BD mutant alleles lack clear plate phenotypes, likely due to the fact that the normal interaction of bromodomains with their substrates involves an interaction with the acetyl moiety (which is compromised) and also the peptide sequence (which is retained). However, the combined BD1&2 mutations conferred lethality (even in WT *GCN5* background, Figure 2A). This demonstrates that the two bromodomains are partially redundant; compromising the acetyl-binding pocket of both bromodomains is needed to confer inviability. We note that redundancy can result from the two bromodomains binding to different ligands; compromising one bromodomain makes Rsc4 reliant on the alternative bromodomain and its ligand(s) to conduct an essential function.

In support of the two bromodomains binding different ligands, we found that *rsc4* Y92A Y93A *gcn5Δ* combinations were lethal, whereas *rsc4* Y225A Y226A *gcn5Δ* combinations grew well (Figure 2A). As above, lethality could result from compromising both BD1 and BD2; BD1 is compromised through mutation of the tyrosine residues and BD2 through the lack of the acetyl group on the H3K14 substrate in the *gcn5Δ* background. Consistent with this notion, synthetic lethality was also observed when the BD1 mutant was combined with the *h3*K14G mutant (Figure 2A). These data implicate BD2 in binding H3K14ac, because mutations in BD2 that prevent the recognition of the acetyl moiety (Y225A, Y226A) are not expected to be exacerbated by the absence of the acetyl group on the substrate (from *gcn5Δ*). In contrast, the synthetic lethality observed by combining a BD1 mutation with *gcn5Δ* implicates an important function for BD1 in binding a ligand(s) distinct from H3K14ac.

An NMR titration experiment indicated that the Rsc4-H3K14ac peptide dissociation constant is approximately 1–2 mM (data not shown). This low inherent binding is not unusual for bromodomains (Hudson et al., 2000; Shen et al., 2007; Sun et al., 2007) and presumably reflects the involvement of additional contacts with the nucleosome in the context of the intact RSC complex. It does, however, limit the choice of binding assay, and we therefore estimated relative binding affinities using biotinylated H3 tail peptides (residues 1–39) that were either acetylated at K14 or unmodified, and were immobilized on streptavidin beads. This analysis revealed that Rsc4(46–334) binds H3K14ac peptides in preference to unmodified peptides (Figure 2B). Consistent with the genetic experiments, the Y92F variant (intact BD2) binds H3K14ac peptides preferentially in the same manner as the wild-type (WT) sequence, whereas preferential binding is abolished with the equivalent mutation in BD2 (Y225F) (Figure 2B). Moreover, a Rsc4(157–321) BD2 derivative that lacked BD1 entirely still demonstrated a preferential interaction with H3K14ac peptides compared to unmodified peptides (Figure 2C). Not surprisingly, given the extensive interactions between BD1 and BD2, peptide binding to the isolated BD2 structure was weaker than to the TBD, and the isolated BD1 was insoluble (data not shown). The binding assay was further used to map the Rsc4 interaction to residues 6–21 of the H3K14ac peptide, with further truncation from the N terminus preventing binding to the intact Rsc4(36–321) (data not shown). Thus, these data indicate that Rsc4 BD2 preferentially binds the H3 tail acetylated at K14 with other interactions also contributing to binding affinity. We also examined whether or not modifications near H3K14 affect H3K14ac recognition. Previous work suggested that H3K9 acetylation does not affect Rsc4 binding (Kasten et al., 2004). However, S10 phosphorylation has previously been linked to H3K14 acetylation (Lo et al., 2000) and had not been examined in our prior studies. We find that S10 phophorylation antagonizes selective binding of Rsc4(36–321) to H3K14ac peptides, raising the possibility that this modification may restrict RSC binding in vivo (Figure 2D).

Structure of Rsc4 BD2 Bound to H3K14ac

To visualize the H3-Rsc4 interaction, we determined the structure of a peptide complex by soaking Rsc4(36–340) crystals in high concentrations (~40 mM) of H3(6–18)K14ac (Table 1). Density for the peptide backbone is not apparent in this 1.7 Å resolution structure but is clearly defined for an acetylated lysine side chain in the BD2 binding site in a conformation that overlaps closely with the structure of a Gcn5 bromodomain ligand complex (Figure 2E). Unfortunately, density was not reliably interpretable beyond the side chain, and it was not possible to determine the path of the bound peptide backbone. Numerous data sets were obtained and structures determined at 1.7–2.3 Å resolution for Rsc4(36–340) cocrystallized or soaked with H3 peptide, but ligand density was always limited to the Kac side chain. The BD1 and BD2 pockets are open in the crystal lattice, although they are each within 6 Å of significant crystal contacts. Because in vitro binding appears normal in the 6–21 H3K14ac peptide but is abolished in a 10–21 peptide (data not shown), it seems likely that lattice contacts prevent secondary interactions that are important for full binding affinity and block the peptide from adopting the bound conformation