Inhibition of Protein Interactions with the β_2 Sliding Clamp of *Escherichia coli* DNA Polymerase III by Peptides from β_2-Binding Proteins†

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ABSTRACT: The sliding clamp of the *Escherichia coli* replisome is now understood to interact with many proteins involved in DNA synthesis and repair. A universal interaction motif is proposed to be one mechanism by which those proteins bind the *E. coli* sliding clamp, a homodimer of the β subunit, at a single site on the dimer. The numerous β_2-binding proteins have various versions of the consensus interaction motif, including a related hexameric sequence. To determine if the variants of the motif could contribute to the competition of the β-binding proteins for the β_2 site, synthetic peptides derived from the putative β_2-binding motifs were assessed for their abilities to inhibit protein−β_2 interactions, to bind directly to β_2, and to inhibit DNA synthesis in vitro. A hierarchy emerged, which was consistent with sequence similarity to the pentameric consensus motif, QL(S/D)LF, and peptides containing proposed hexameric motifs were shown to have activities comparable to those containing the consensus sequence. The hierarchy of peptide binding may be indicative of a competitive hierarchy for the binding of proteins to β_2 in various stages or circumstances of DNA replication and repair.

Different DNA polymerases are responsible for specific processes during the replication, recombination, and repair of chromosomal DNA in bacteria, and their individual roles appear to be mediated in part by their interactions with different, but overlapping, sets of accessory proteins. Nevertheless, most of the eubacterial DNA polymerases appear to interact with at least one common protein partner, originally recognized in *Escherichia coli* as the β subunit of the replicative DNA polymerase III (Pol III) holoenzyme (*1*, *2*, *5*). The β subunit forms a stable head-to-tail homodimer giving rise to a donut-shaped structure (*3*) that is able to encircle and slide along duplex DNA (*4*). The earlier view of the limited role of the β dimer in DNA metabolism as the processivity factor or "sliding clamp" associated only with Pol III holoenzyme has thus undergone significant revision in recent years.

The β dimer (β_2) interacts with the α subunit (encoded by *dnaE*) within the *E. coli* Pol III holoenzyme. In Gram-positive bacteria, the related PolC subunit appears to have a similar role to α and also interacts with the cognate β_2 (*6*). Being a protein that encircles duplex DNA, β_2 must be opened and closed at primer termini, and this is the role of a subcomplex of Pol III holoenzyme subunits called the γ clamp loader complex (*7*, *8*). The single δ subunit in the γ complex interacts with β_2 to open the locked homodimer at one of the subunit interfaces to allow its assembly onto double-stranded DNA (*9*).

Several other polymerases including Pol II (or PolB), Pol IV (or DinB), and Pol V (or UmuD/C) are capable of error-prone replication past sites of DNA damage (translesion synthesis) (*10*). All three of these polymerases also bind to β_2 (*11*−*14*). Furthermore, DNA polymerase I, MutS (a mismatch recognition protein), and DNA ligase have also been shown to interact with β_2 (*2*).

The β_2 protein may, in addition, be involved in early events in chromosome replication (*15*). DnaA, the replication initiator protein, recognizes the origin of replication (*oriC*) to initiate replication (*16*, *17*). Prevention of unprogrammed initiation of additional cycles of replication involves the sequestration of hemimethylated *oriC* by SeqA protein, binding of DnaA to "diversion" sites within the *datA* locus, and conversion of active ATP-DnaA to its inactive ADP-bound form (*17*−*19*). The recently identified Hda protein, in the presence of a β_2−DNA complex, appears to mediate this regulatory inactivation of DnaA (*20*). It had earlier been suggested that this occurs via interaction of ATP-DnaA at *oriC* with DNA-bound β_2, mediated physically by an unidentified factor (IdaB) that now appears to be Hda (*15*, *21*).

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The utilization of the versatile β_2 sliding clamp by so many polymerases, as well as its interaction with the δ subunit of the γ complex, Hda (*22*), and other proteins, raises questions about the positions and interplay of binding sites on the β_2 ring. For example, the α and δ subunits of Pol III holoenzyme are thought to have overlapping interaction sites on the β_2 molecule (*23*). We recently identified a pentapeptide motif with consensus sequence QL(D/S)LF apparently involved in the interaction of many proteins with β_2. Short segments of DnaE (α subunit of Pol III), PolB, DinB, UmuC/D, and MutS with this sequence were observed to bind β_2 using a yeast two-hybrid system, and synthetic peptides containing the consensus sequence inhibited $\alpha-\beta_2$ and $\delta-\beta_2$ interactions in vitro (*24*). Since that report, the putative β_2-binding motif within DinB (the carboxyl-terminal six amino acids) has been shown to be essential for its association with β_2 (*14*). Moreover, when the putative β_2-binding regions of the repair polymerases PolB, DinB, and UmuC were modified by mutagenesis, in vivo translesion synthesis activity was lost (*25*). Two crystal structures of the fragments of DinB (Pol IV) polymerase cocrystallized with β_2 were reported recently (*26*, *27*). The first, of the β_2 ring in complex with two molecules of the C-terminal "little finger" domain (residues Val243−Leu351 of DinB) at 1.90 Å, shows a symmetrical arrangement where the DinB domain occupies both binding sites on the β_2 dimer (*26*). The other structure, at 1.65-Å resolution, has a single molecule of the C-terminal 16-residue peptide of DinB occupying one of the β_2 sites (*27*). The conformations of the structured residues present in both (corresponding to Arg345−Leu351) appear to be very similar in the two complexes, and the binding site on β_2 is the same as that occupied by the δ subunit (*26*, *27*). The 16-residue peptide at concentrations around 10 μM was also shown to inhibit β_2-dependent in vitro DNA replication promoted by both DinB and the α subunit of Pol III (*27*).

Bioinformatic analysis of families of proteins related to those described above has been used to identify putative β_2-binding sites in several other families of probable DNA polymerases, DinB2, DinB3, and DnaE2, and in members of the Duf72 (InterPro IPR002763) family of proteins of unknown function (*28*).

Undoubtedly, many factors determine which molecules interact with β_2 at a particular time at a particular site on DNA that is undergoing replication or repair. To gain some insight into the competitive events that take place, we have investigated the β_2-binding motifs derived from various β_2-interacting partners including Hda. We have measured the ability of synthetic peptides based on the putative β_2-binding sequences to associate directly with β_2 and their ability to compete with the binding of β_2 to the δ and α subunits of Pol III holoenzyme. We have recently presented evidence for the physical interaction of Hda with β_2 and shown that it is mediated through a hexapeptide motif, QL(S/P)LPL, conserved among members of the Hda family from many bacterial species (*22*).

MATERIALS AND METHODS

Protein Expression and Purification. The *E. coli* β_2 sliding clamp was prepared as described by Oakley et al. (*29*). The bacteriophage T7 promoter plasmids pET-δ and pET-δ' (*30*), used for overproduction of the δ and δ' subunits, respectively, of *E. coli* Pol III holoenzyme in strain BL21(DE3)/pLysS (*31*), were generous gifts from Dr. Mike O'Donnell. The α subunit of Pol III was overproduced in strain BL21(DE3) (*31*) containing plasmid pND517. This plasmid contains the complete *E. coli dnaE* gene, with an artificial ribosome-binding site, in the *tac* promoter vector ptac-85 (*32*). Details of the construction of pND517, as well as the methods adapted from the published procedures for isolation of highly purified samples of α, δ, and δ', and their characterization by SDS−PAGE and ESI-MS, are given in the Supporting Information. Concentrations of proteins were estimated using the BCA Protein Assay (Pierce, Rockford, IL) with bovine serum albumin as the standard.

Peptide Synthesis and Purification. Several peptides were synthesized and purified as described (*10*). These were PepDnaE-n, pepDnaE-1, pepDnaE-4, consensus-1, consensus-2, pepdelta-n, and the control peptide. The consensus-1 and consensus-2 peptides contain the two versions of the consensus pentamer, QLDLF and QLSLF, respectively (see Table 1).

Other peptides were synthesized by the Fmoc protection strategy using a chlorotrityl polystyrene resin (Pepchem), except for the amino-terminal acetylated peptides (see Table 2). These peptides, which also contained a carboxyl-terminal 2-aminoethylamide moiety, were prepared using an ethylenediamine trityl-polystyrene resin (NovaBiochem). Amide couplings were carried out in DMF[1] for 10 min with preactivated Fmoc amino acids (3 equiv relative to the resin loading) using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (2.7 equiv) and DIPEA (4 equiv). The Fmoc group was cleaved using two successive treatments (1 min each) with a 1:1 (v/v) mixture of piperidine and DMF. For attachment of the first Fmoc amino acid to the chlorotrityl polystyrene resin, DIPEA (1.5 equiv) was added to the amino acid derivative (1.5 equiv) in anhydrous DCM (approximately 8 mL/g of resin). The solution was then added to the resin, and the suspension was stirred. After 5 min, further DIPEA (3 equiv) was added. After another 45−60 min, methanol (1 mL/g of resin) was added, and reaction was allowed to proceed for 15 min before the resin was drained, washed three times with a mixture (1:1, v/v) of DCM and DMF and six times with DCM, and then dried in vacuo.

The resin loaded with the first amino acid was suspended in a "zero-gravity" mixture of chloroform and DMF (ca. 1:1). Aliquots of this suspension (each about 30−50 mg of resin) were transferred to the wells in a 96-well block, while the suspension was being kept homogeneous by gentle shaking. At the end of the peptide synthesis, the Fmoc groups were removed from the last amino acids and the resins were washed 6 times with DMF and 10 times with DCM and dried by a stream of air (suction). They were then treated with a cleavage cocktail containing trifluoroacetic acid, DCM, water, and triisopropylsilane, typically in the ratio (v/v) 14:4:1:1 (1 mL/well). After cleavage for a period of 2 h, the suspensions were filtered and the resins were washed twice with the cleavage cocktail. The resulting filtrates were analyzed by mass spectrometry and analytical HPLC, and

[1] Abbreviations: DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; SPR, surface plasmon resonance.

Table 1: Inhibition of $\alpha-\beta_2$ and $\delta-\beta_2$ Interactions by Peptides[a]

peptide[b]	sequence	protein family	$\alpha-\beta_2$ binding IC_{50} (μM)[c]	$\delta-\beta_2$ binding IC_{50} (μM)[c]
Series 1. Peptides with Putative β_2-Binding Pentameric Motifs from DnaE and Variants				
pepDnaE-n	IG QADMF GV	DnaE1	66 ± 6	120 ± 14
pepDnaE-1	IG QLDMF GV	DnaE1	3.6 ± 0.9	7.2 ± 3.7
pepDnaE-4	IG QADMA GV	DnaE1	>500	>500
Series 2. Peptides with Putative β_2-Binding Pentameric Motifs from *E. coli* β_2-Binding Proteins				
consensus-1	IG QLDLF GV		0.6 ± 0.2	2.4 ± 0.6
consensus-2	IG QLSLF GV		2.3 ± 1.1	5.6 ± 3.9
pepMucB-n	EA QLDLF DS	UmuC	1.0 ± 0.5	4.0 ± 1.6
pepUmuC-e	IG QLNLF GV	UmuC	8.1 ± 0.1	17 ± 4
pepPolB-e	IG QLGLF GV	PolB2	11 ± 3	6.3 ± 1.7
pepMutS-e	IG QMSLL GV	MutS1	26 ± 1	18 ± 5
control	IL LDFGQ VG		>500	>500
Series 3. Peptides with Putative β_2-Binding Hexameric Motifs from *E. coli* β_2-Binding Proteins				
pepHda-n	PA QLSLPL YL	DnaA2	1.1 ± 0.5	2.1 ± 1.0
pepHda-e	IG QLSLPL GV	DnaA2	5.0 ± 0.7	5.8 ± 2.7
pepDinB-e	IG QLVLGL GV	DinB1	24.5 ± 0.7	15.8 ± 2.6
Series 4. Peptides of the β_2-Binding Region of the δ Subunit				
pepdelta-n	SLC QAMSLF AS	HolA	22 ± 4	485 ± 14
pepdelta-e	IG QAMSLF GV	HolA	22 ± 9	205

[a] As measured in plate assays as described in the Materials and Methods. [b] Positions 1 and 2 and the penultimate and last positions of the sequences of these peptides utilized the flanking dipeptides from the sequence of the α subunit of Pol III and are denoted by the suffix "-e". The exceptions are pepMucB-n and pepHda-n, which consist entirely of the native sequences (suffix "-n"), from MucB and Hda proteins, respectively. [c] The IC_{50} values represent the molar concentration of the peptide required to inhibit the interactions by 50%. Errors are standard errors of the mean of at least two independent measurements.

Table 2: Summary of Binding and Inhibition Parameters Obtained by SPR and in Vitro DNA Replication Assays

peptide	sequence	BIAcore[a] K_D (μM)	IC_{50}(B)[b] (μM)	IC_{50}(R)[c] (μM)
pepDnaE-n	IG QADMF GV	2.70	n.d.[d]	n.d.
pepDnaE-1	IG QLDMF GV	0.56	1.01	70
pepDnaE-4	IG QADMA GV	>10	n.d.	>2500
consensus-1	IG QL DLF GV	0.78	0.50	24
consensus-2	IG QLS LF GV	0.80	0.55	25
pepMucB-n	EA QLDLF DS	0.51	0.58	15
pepUmuC-e	IG QLNLF GV	n.d.	1.20	80
pepPolB-e	IG QLGLF GV	0.85	1.45	80
pepHda-e	IG QLSLPL GV	0.45	0.19	9
pepHda-n	PA QLSLPL YL	0.38	0.24	2.2
AcpepNH_2consensus-1	Ac-IGQLDLFGV-$NHCH_2CH_2NH_2$	0.63	n.d.	n.d.
AcpepNH_2DnaE-4	Ac-IGQADMAGV-$NHCH_2CH_2NH_2$	>10	n.d.	n.d.
AcpepNH_2Hda-n	Ac-PAQLSLPLYL-$NHCH_2CH_2NH_2$	0.40	n.d.	n.d.

[a] Synthetic peptides were biotinylated (through their amino terminus or carboxyl-terminal ethyleneamine), captured on streptavidin-coated flow-cell surfaces of a BIAcore chip, and assayed directly for binding to β_2. These experiments generated the dissociation equilibrium constant (K_D). [b] The unmodified peptides were mixed at various concentrations with β_2 (0.1 μM) in solution and assessed for their ability to inhibit the binding of β_2 to biotinylated and immobilized consensus-1 peptide on the surface of a BIAcore chip. The IC_{50}(B) value is the concentration of the peptide that results in lowering of the BIAcore response by 50%. [c] The unmodified synthetic peptides were added directly to a preassembled in vitro DNA replication reaction, which was then treated at 30 °C for 10 min. The IC_{50}(R) value is the concentration of the peptide that results in inhibition of the observed extent of DNA synthesis by 50%. [d] n.d. = not determined.

the samples were concentrated to dryness in vacuo using a Genevac freeze dryer. The dried crude peptides were washed three times with diethyl ether and purified by preparative reverse-phase HPLC. All peptides were >95% pure as assessed by analytical HPLC, and mass spectrometric analyses were consistent with the calculated values.

Plate-Binding Assays. The $\alpha-\beta_2$- and $\delta-\beta_2$-binding inhibition assays (performed in microtiter plates at 25 °C in 20 mM Tris-HCl buffer at pH 7.5, 0.1 mM EDTA, and 10 mM $MgCl_2$) were conducted as described by Dalrymple et al. (*24*), with the exception that the buffer in the $\alpha-\beta_2$ assay contained 10 mM $MnCl_2$ instead of 10 mM $MgCl_2$; this assay was observed to be 4−5-fold more sensitive when $MnCl_2$ was used. A $\delta-\delta'$-binding assay was used as a control. This assay was constructed on the same principle as that used for the $\alpha-\beta_2$- and $\delta-\beta_2$-binding studies, with the following changes: the δ subunit was adsorbed onto the plate at 2 μg/mL, and the synthetic peptides were pretreated with the δ' subunit (at 5 μg/mL) for 90 min before being transfered to the δ subunit-coated plate. Binding of δ' to δ was detected with a rabbit antiserum to the δ' subunit and a sheep anti-rabbit Ig-horseradish peroxidase conjugate.

Surface Plasmon Resonance (SPR). Purified peptides (10 μg each) in 75 mM sodium borate at pH 8.5 (250 μL) were reacted overnight at room temperature with 50 μL of a 20 mg/mL solution of the sulfosuccinimidyl ester of 6-((6-((biotinoyl)amino)hexanoyl)amino)hexanoic acid (biotin-XX SSE; Molecular Probes, Eugene, OR) in dimethyl sulfoxide. The reaction mixtures were separated by HPLC (Shimadzu, Kyoto, Japan) using a Brownlee C18 cartridge (Applied

Biosystems Inc., Foster City, CA) and a gradient of 6−65% acetonitrile in 0.1% trifluoroacetic acid delivered at 0.5 mL/min during 40 min. The biotinylated peptides, which eluted later than the biotinylated linker and free peptide, were collected, vacuum-dried, and then dissolved in water. They were assessed in the plate assays to ensure retention of inhibitory activity.

SPR experiments were conducted at 25 °C with a BIAcore 2000 instrument using streptavidin-derivatized flow-cell surfaces (Biacore AB, Uppsala, Sweden). Free peptide solutions were prepared in 20 mM Tris-HCl at pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, and 0.005% v/v Tween-20, and β_2 was diluted into the same buffer from a 17.5 μM stock solution in a similar buffer containing 20% (v/v) glycerol. In the β_2-binding studies, the biotinylated peptides were loaded onto flow-cell surfaces such that the interaction with 0.5 μM β_2 produced a net signal (after background subtraction) of 100−400 response units (RU; estimated at 1.25−5 fmol of β_2/mm^2 based on 1000 RU per ng/mm^2; Biacore). Upon completion of the injection of β_2, the response quickly returned to baseline at flow rates of 10 and 50 μL/min; therefore, regeneration buffers were not required. Flow cell 1 was not derivatized and was used as a control surface for background subtraction. Checks showed that binding responses were not limited by mass transfer. Values of dissociation constants (K_D) were determined using the RU values obtained at steady state with 15 different concentrations of β_2 (0.01−5 μM, in duplicate at random) for each biotinylated peptide attached to the flow-cell surface. After subtraction of the background response, the data were fit to the 1:1 Langmuir model using BIAEvaluation (Biacore). The association and dissociation phases were both too fast to obtain reliable estimates of the rate constants.

For solution affinity analyses, a flow-cell surface was prepared with a higher loading of the biotinylated consensus-1 peptide so that it was capable of generating 700−1200 RU on injection of 0.5 μM β_2, representing an estimated 9−15 fmol/mm^2. Loading with the same amount (in pmol) of pepDnaE-4 generated a surface used as a negative control. This peptide does not interact with β_2, and a response from the interaction with solutions of β_2 could not be observed. In all data manipulations, the responses from this surface were subtracted from those from the consensus-1 surface. A calibration curve of RU values generated at different concentrations of β_2 (10−100 nM) was developed for the immobilized consensus-1 peptide. To determine the inhibitory effect of free peptide, β_2 (100 nM) was pretreated for 10 min with various concentrations of underivatized peptide (0.01−4.5 μM, in duplicate) to form a complex with β_2, which was then passed over the flow-cell surfaces. The amount of free uncomplexed β_2 remaining was determined from the calibration curve. Plots of the concentration of the uncomplexed (free) β_2 against the concentrations of inhibitory peptides were used to determine IC_{50} values; these represent the concentrations of peptides in solution required to prevent binding of half of 100 nM β_2 to the surface.

In Vitro DNA Replication Assays. ABC-primosome assays (*33*, *34*), which monitor complementary strand synthesis using a phage M13-derived single-stranded DNA template containing a DnaA protein-binding site from the γ2 origin of plasmid R6K (M13-A site DNA), were carried out as described by Oakley et al. (*29*). Recognition of the origin of replication in the single-stranded DNA-binding protein (SSB)-coated template by the DnaA replication initiator protein leads to the loading of the DnaB helicase from the $DnaB_6$•$DnaC_6$ complex, RNA priming by the DnaG primase, and primer extension by DNA Pol III holoenzyme.

The extent of Pol III holoenzyme-dependent DNA synthesis at 30 °C during a 10-min period in a total volume of 25 μL was monitored by the measurement of incorporation of [^{3}H]-TMP into acid-precipitable products. Assembly of the active holoenzyme from limiting amounts of DNA Pol III* (holoenzyme lacking β_2) and excess purified β_2 (25 ng, 308 fmol) led to the duplication of about 60% of the template under these conditions. Stocks of peptides were prepared in water, and all subsequent dilutions were made either in water or in a β dilution buffer (50 mM Tris-HCl at pH 7.6, 20% v/v glycerol, 1 mM DTT, and 1 mM EDTA). Diluted peptides were added directly to preassembled reaction mixtures at 0 °C, before DNA synthesis was initiated at 30 °C. Results are presented as the inhibition (%) relative to a control reaction that contained no added peptide.

Molecular Modeling of Peptides Bound to β. The following protocol was followed to model the structure of the pentameric peptide QLSLF bound to the monomeric β subunit. The starting structure was the segment Ala70-Phe74 (sequence AMSLF) of the δ subunit (B chain) in complex with the monomeric β subunit (A chain) from the crystal structure of this complex, PDB 1JQL (*35*). First, Insight II version 98.0-Homology (Accelrys, San Diego, CA) was used to mutate the B chain Ala70 to Gln and Met71 to Leu, selecting rotamers such that the side chains were directed toward the A chain. After hydrogen atoms and capping groups were added, this structure was energy minimized with Discover version 2.98 (Accelrys) using the CVFF force field and a distance-dependent dielectric function, keeping fixed the backbone of the A chain and all heavy atoms of the A chain side chains not interacting with the B chain. Next, rigid body docking was performed with the B chain portion of the minimized structure on the A chain portion of the minimized structure using 3D-Dock (*36*). To make the grid spacing sufficiently small, residues 1−121 of the A chain were neglected during the docking. Default values were used for the parameters. The grid spacing was 0.7 Å. The docked solutions were first ranked with an empirical pair potential score corresponding to the empirically derived likelihood of the occurrence of a trans-interface pair of two residue types (*37*) and then filtered such that residues Phe at position 5 and Leu at position 4 of the peptide (i.e., the B chain) were no more than 4.5 Å from Val247 and Met362, respectively, of the A chain. The model top ranked by the pair potential score was selected as the structural model of the pentamer QLSLF bound to the A chain. After hydrogen atoms were added, bad contacts in this model were relieved by a further cycle of energy minimization using Discover version 2.98, keeping fixed the backbones and A chain heavy atoms of the residues not interacting with the peptide, resulting in the final model. A similar procedure was used to model the structure of the hexameric peptide QLSLPL bound to β. Here, the starting structure was the segment Gln346-Leu351 (sequence QLVLGL) of the little finger domain of Pol IV (D chain) and a monomer of the β subunit (A chain) from the crystal structure of the complex, PDB 1UNN (*26*). LIGPLOT (*38*) was used to plot the interactions between

the peptides and β in the constructed models (see the Supporting Information), and X-Score version 1.1 (*39*) was used to calculate the binding affinity of the peptide to β_2.

RESULTS

Inhibition of $\alpha-\beta_2$ and $\delta-\beta_2$ Interactions by β-Binding Peptides. Four sets of synthetic peptides were first examined for their ability to compete with the α and δ subunits of *E. coli* Pol III holoenzyme for binding to β_2, as assessed in simple plate assays (see Table 1 and representative data in Figure 1). With some exceptions, the putative β-binding penta- or hexapeptides were flanked at their amino and carboxyl termini by the flanking dipeptides that occur in the α subunit sequence. This was designed to exclude the potential contribution of different flanking amino acids from the various protein sequences, so as to allow direct comparison between the putative β_2-binding peptides. Peptides utilizing the flanking dipeptides from the α subunit are denoted by the suffix "-e". Peptides that consist entirely of native sequences are denoted by the suffix "-n".

Sequences of peptides in the first series (Table 1) were based on the putative pentameric β-binding sequence from the α subunit (QADMF). The set did not include a peptide from a second β-binding site recently discovered by Lopez de Saro et al. (*40*). This second site is located at the carboxyl terminus of the α subunit. Its sequence, QVELEF, is reminiscent of the hexameric β-binding sequences of DinB1 and Hda.

PepDnaE-n, though a convincing inhibitor of the $\alpha-\beta_2$ and $\delta-\beta_2$ interactions, had moderate inhibitory activity, and substitution with Ala at positions 1, 3, 4, and 5 resulted in markedly poorer competition (*24*). On the other hand, PepDnaE-1 (pentamer sequence QLDMF) had improved inhibitory activity toward both interactions, presumably because it is more similar to the β-binding consensus pentamer QL(D/S)LF. This consensus sequence had been derived from bioinformatic analysis of eubacterial β_2-binding proteins; the amino acid at each position is that observed most frequently (*24*, *28*).

The second series of peptides (Table 1) included the two that contain these consensus sequences. The peptides containing the two versions of the consensus motif QL(D/S)LF, consensus-1 and consensus-2, were already known to be better competitors than pepDnaE-n in the binding assays (*24*). The "control" peptide (Table 1) was a scrambled version of the consensus-1 peptide. These peptides were compared to four natural variants of the consensus sequence (Table 1) with respect to their inhibition of the $\alpha-\beta_2$ (Figure 1A) and $\delta-\beta_2$ interactions (Figure 1B). Values of IC_{50} (50% inhibition of binding) are given in Table 1. All four substitutions in these examples decreased the inhibitory activity of the peptides.

Some DinB (Pol IV) polymerases appear to possess a hexameric rather than a pentameric β_2-binding sequence, and this led to the identification of a hexapeptide motif in Hda (*22*). Indeed, two groups (*26*, *27*) have independently determined the crystal structures of the hexameric motif at the C terminus of larger DinB fragments in complex with β_2. In the present paper, peptides containing the hexameric motifs, pepHda-e and pepDinB-e, were shown also to be inhibitors of $\alpha-\beta_2$- and $\delta-\beta_2$-binding (parts A and B of Figure 1, Table 1). Furthermore, the putative Hda hexameric motif was as effective as the consensus peptides bearing the pentameric consensus motif.

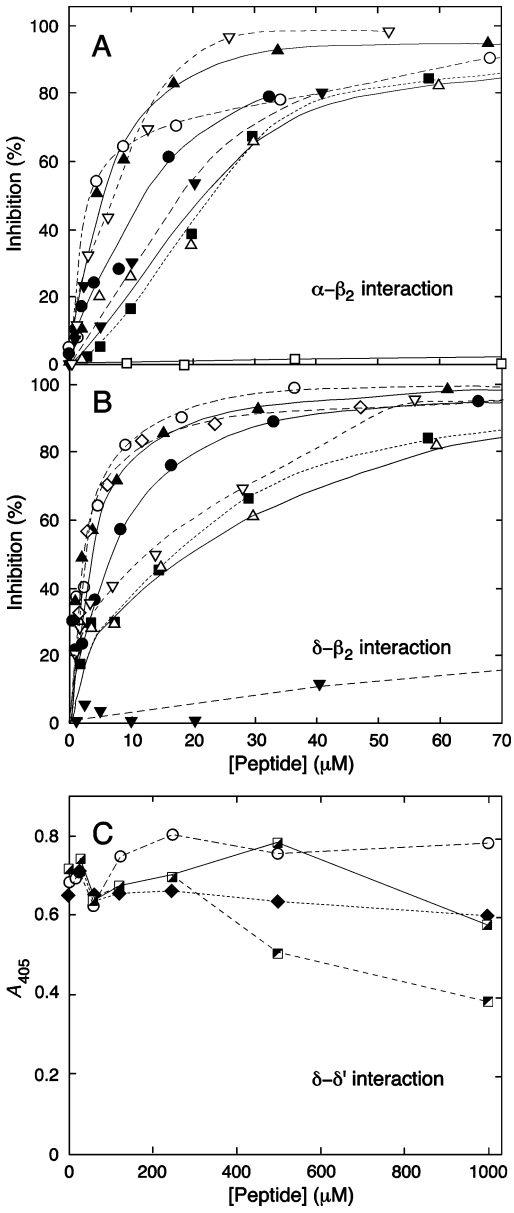

FIGURE 1: Representative data showing inhibition of protein−protein interactions in plate assays by synthetic peptides. (A) Inhibition of the $\alpha-\beta_2$ interaction. (B) Inhibition of the $\delta-\beta_2$ interaction. Peptides from series 2−4 (Table 1) were titrated into plate assays that detect the protein−protein interactions (see the Materials and Methods for details). Data are expressed as inhibition (%) of binding of β_2 to α or δ relative to the values obtained in the absence of the peptide and are derived from single experiments. The lines have no theoretical significance. Average values of IC_{50} determined from similar plots from two or more separate experiments with each peptide are given in Table 1. (C) Lack of effect of peptides on $\delta-\delta'$ (control) interaction. Data are presented as absorbance units (A_{405}) to demonstrate the lack of significant inhibition of binding of δ' to δ at peptide concentrations of up to 1 mM. Sequences of the peptides are given in Tables 1 and 2: pepDnaE-n (◪), pepDnaE-1 (◩), pepDnaE-4 (□), consensus-1 (◆), consensus-2 (○), pepMucB-n (◇), pepUmuC-e (▽), pepPolB-e (●), pepMutS-e (■), pepHda-e (▲), pepDinB-e (△), and pepdelta-e (▼).

The δ subunit forms a stable complex with β_2 (*11*), and residues Met71, Leu73, and Phe74 in the hexapeptide binding region of δ are known to interact directly with β_2 from the

crystal structure of a complex of the δ subunit with a monomeric version of β (*35*). Mutagenesis of these residues in the δ subunit indicates their important contributions in this interaction (*41*). A peptide containing the β_2-binding region of δ, QAMSLF (pepdelta-e), was tested to see if it was a superior inhibitor of the $\alpha-\beta_2$ and $\delta-\beta_2$ interactions. As seen in parts A and B of Figure 1 and Table 1, this was not the case.

Several peptides, pepMucB-n, pepHda-n, and pepdelta-n, were synthesized with the naturally occurring di- (or tri-) peptides flanking the putative β_2-binding sequences to determine the effect of the flanking sequences. These peptides behaved similarly to their "DnaE" flanked counterparts as competitors in both binding assays (Table 1). To ensure that peptides did not interfere with protein−protein interactions in some nonspecific way, they were assessed in a similarly constructed $\delta-\delta'$-binding assay. None of the peptides appeared to inhibit this interaction; representative data are shown in Figure 1C.

Characterization of the Binding of β_2 to Peptides by SPR. SPR was used to directly assess the ability of β_2 to bind to several peptides. Peptides were attached through their amino termini via a 12-carbon chain to biotin and then bound to streptavidin-coated surfaces of a BIAcore flow cell. Sensorgrams clearly showed the binding of β_2 to the biotinylated peptides, with rapid association and dissociation rates (Figure 2A). Response measurements (RU) were taken at R_{max} (i.e., at the plateau of the binding curve achieved at steady state), and the background (flow cell 1, with no peptide attached) was subtracted (Figure 2B). The background responses were large because of the higher refractive index of the analyte solutions containing high concentrations of β_2 and significant amounts of glycerol (see the Materials and Methods). Nevertheless, reliable binding isotherms were obtained that gave good fits of the steady-state data to a 1:1 Langmuir-binding model, described by the values of the dissociation constants, K_D, given in Table 2. Examples of isotherms are shown in Figure 3, and data for the other peptides are among the Supporting Information.

The sensorgrams in Figure 2B show, in concordance with the plate-binding assays (Table 1), that pepDnaE-4 has little capacity to bind β_2. With β_2 at 5 μM, R_{max} with pepDnaE-4 was 10-fold lower than with peptide consensus-1. Also consistent with the earlier assays, pepDnaE-n gave the highest value of K_D (2.7 μM), while those peptides that were found to be better inhibitors in the $\alpha-\beta_2$ and $\delta-\beta_2$ plate assays gave values in the range 500−850 nM (Table 2). PepUmuC-e was not assessed in this assay because its sequence and behavior were similar to pepPolB-e. PepHda-e and pepHda-n bound most avidly of all, giving K_D values of ~400 nM.

Those peptides with natural flanking sequences, pepMucB-n and pepHda-n, showed very similar behavior to their DnaE-flanked counterparts, consensus-1 and pepHda-e, respectively. To show that the orientation of the peptide with respect to the surface did not affect β_2 binding, analogues with the sequences of pepDnaE-4, consensus-1, and pepHda-n were synthesized with an acetylated amino terminus and a 2-aminoethylamide extension at the carboxyl terminus through which the biotinylated linker was attached; these peptides were named AcpepNH_2DnaE-4, AcpepNH_2consensus-1, and AcpepNH_2Hda-n, respectively. The K_D values of the

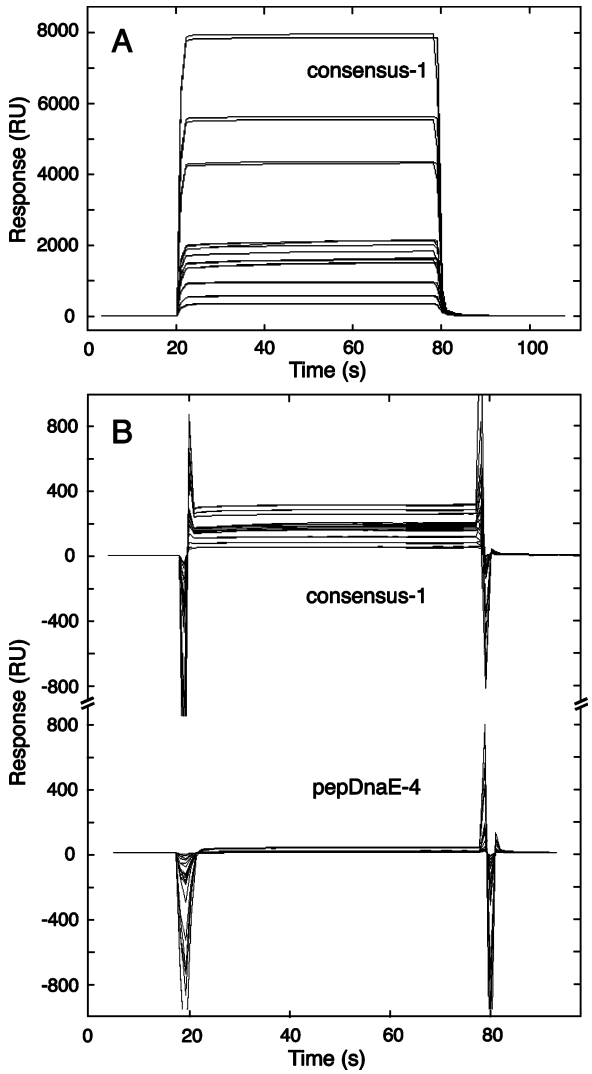

FIGURE 2: SPR studies of the binding of β_2 to immobilized peptides. Biotinylated peptides were captured on flow-cell surfaces via immobilized streptavidin. A total of 15 concentrations of β_2 between 0.01 and 5 μM were injected in duplicate (at random) over the flow-cell surfaces at a rate of 10 μL/min, at 25 °C, and sensorgrams were recorded. (A) Overlay of the uncorrected sensorgrams of duplicate injections (1 min each) of β_2 solutions over a surface bearing the consensus sequence peptide, consensus-1 (Table 1). Concentrations of β_2 increase from the lower to upper traces. A large proportion of the observed response results from the background refractive index change because of high concentrations of β_2 and glycerol in the analyte solution. (B) Background (flow cell 1)-subtracted sensorgrams derived from the (near) simultaneous injections of the same solutions of β_2 over the flow cells containing similar amounts of immobilized peptides consensus-1 (top) and pepDnaE-4 (bottom), under the same instrumental conditions. No attempt was made to correct for the delay (1.09 s) between the time of delivery of the analyte solution to the flow cell 1 (background) and the other flow cells. When the background is significant, this delay gives rise to the large apparent fluctuations at the beginning and end of injections.

complexes of β_2 with each of these peptides were similar to those for peptides bound to surfaces in the original orientation (Table 2, parts B and C of Figure 3).

Relative Competitive Behaviors of Peptides versus the Consensus Sequence. Affinity solution studies of the peptides using SPR allowed the assessment of the ability of the unmodified peptides to bind β_2 in solution. β_2 (at 100 nM) was treated with increasing concentrations of a competing

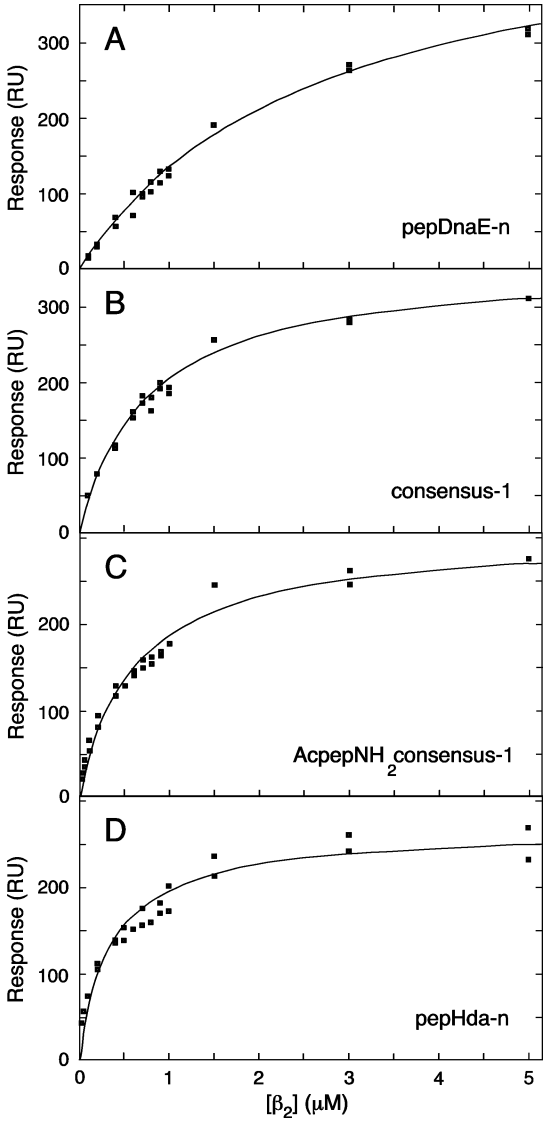

FIGURE 3: Representative data showing binding isotherms for the interaction of β_2 to immobilized peptides (sequences are given in Table 1), studied by SPR. (A) pepDnaE-n. (B) consensus-1 (see Figure 2A). (C) AcpepNH_2consensus-1. (D) pepHda-n. A total of 15 concentrations of β_2 between 0.01 and 5 μM were injected in duplicate (at random) over flow-cell surfaces at a rate of 10 μL/min, at 25 °C. The instrument response (RU) were measured at equilibrium and then corrected for the response obtained with the underivatized control surface. Data were fit to a 1:1 Langmuir-binding model using BIAEvaluation (Biacore). Solid lines were calculated using the values of K_D given in Table 2.

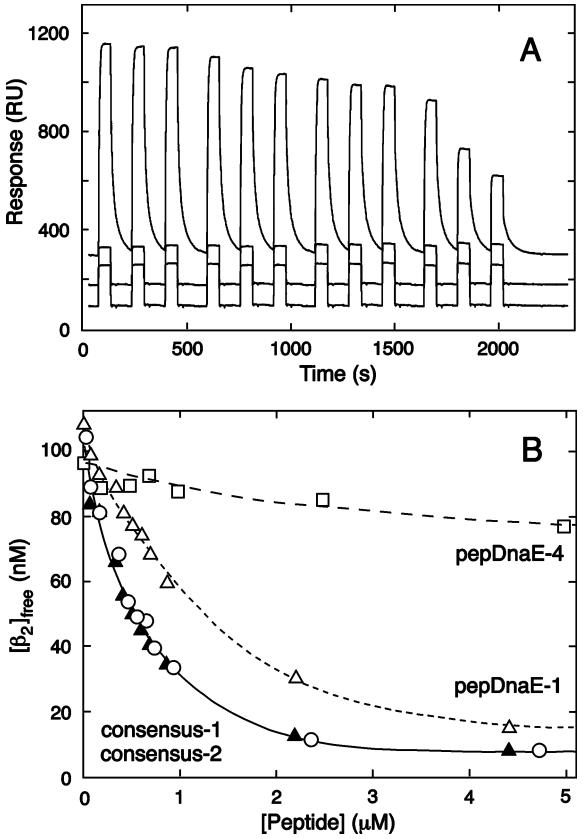

FIGURE 4: Representative data for competition affinity analyses of the effects of peptides on the binding of β_2 to immobilized consensus-1 peptide. Equivalent amounts of biotinylated consensus-1 and pepDnaE-4 peptides were immobilized on the streptavidin-coated surfaces in separate flow cells of a BIAcore chip. Solutions of β_2 (100 nM) that had been pretreated with increasing concentrations of an unmodified competitor peptide were passed at 25 °C in 1-min injections (flow rate = 10 μL/min) through these two flow cells and a control cell whose surface had not been modified. (A) Sensorgrams obtained with the consensus-1 peptide at concentrations of 0−5 μM. Flow-cell surfaces: consensus-1, pepDnaE-4, and control from the top to bottom, respectively. (B) Binding curves plotted as the residual concentration of β_2 available to bind to biotinylated and immobilized consensus-1 peptide against the concentrations of soluble competing pepDnaE-1 (△), pepDnaE-4 (□), consensus-1 (▲), and consensus-2 (○). Data points represent average values from the duplicate experiments. The lines have no theoretical significance. Sequences of the peptides and values of IC_{50} determined from these and similar data are given in Table 2.

peptide in solution and passed over an underivatized surface and flow-cell surfaces coated with high levels of biotinylated pepDnaE-4 or consensus-1. Figure 4A shows typical sensorgrams recorded during sequential injections of increasing amounts of competing peptide (in the presence of 100 nM β_2). Decreasing amounts of β_2 binding were observed in the flow cells containing immobilized consensus-1, whereas the nonderivatized surface and the immobilized DnaE-4 surface did not bind β_2 and were therefore unaffected by the increasing concentrations of the competing peptide.

In a representation of typical data, Figure 4B shows inhibition curves generated by competition of immobilized consensus-1 with soluble pepDnaE-1, pepDnaE-4, consensus-1, and consensus-2. IC_{50} values determined for several peptides in this way are given in Table 2. The data revealed a clear hierarchy. The pepHda-e peptide appeared to be the best competitor (IC_{50} = 190 nM), and the consensus peptides gave IC_{50} values of ~500 nM. The peptide variants of the consensus sequence required 1 μM or more peptide in solution to compete with immobilized consensus-1. Again, pepMucB-n and pepHda-n (which have native flanking dipeptides) showed very similar behavior to their DnaE-flanked counterparts.

Inhibition of in Vitro DNA Synthesis by β_2-Binding Peptides. Many of the peptides were assessed for their ability to inhibit the in vitro replication of a single-stranded phage DNA to its duplex replicative form by a reconstituted replisome. The holoenzyme was reconstituted from separately purified Pol III* (complex of $[\alpha\epsilon\theta]_2\tau_2\gamma\delta\delta'\chi\psi$) and β_2.

Apparent from data in Figure 5, pepDnaE-4 had little inhibitory effect, while the other peptides inhibited DNA synthesis to varying degrees. Consensus-1 and consensus-2 peptides had IC_{50} values near 25 μM. As in the previous

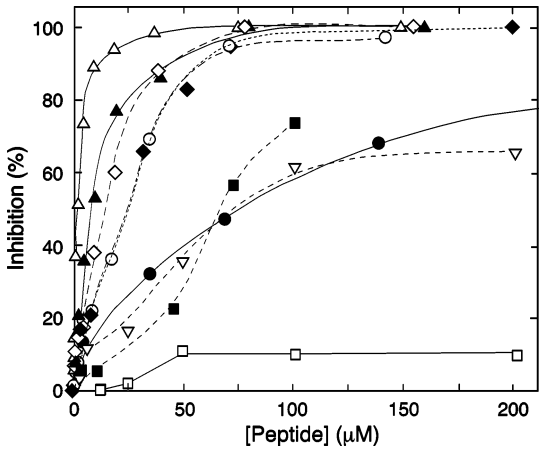

FIGURE 5: Inhibition of in vitro DNA replication by synthetic peptides. Selected peptides were added at various concentrations into ABC primosome reactions (25 μL) containing 220 pmol (as the nucleotide) of template DNA, 20 units of Pol III*, and 308 fmol of β_2 (*29*). Diluted peptides were added directly to the preassembled reaction mixture at 0 °C. Incorporation of [^{3}H]-TMP was measured after 10 min at 30 °C. Data are presented as the inhibition (%) of a reaction containing no added peptide (120−140 pmol of total nucleotide incorporation); the lines have no theoretical significance. Values of IC_{50} determined from these plots and sequences of the peptides are given in Table 2: pepDnaE-1 (■), pepDnaE-4 (□), consensus-1 (◆), consensus-2 (○), pepMucB-n (◇), pepUmuC-e (▽), pepPolB-e (●), pepHda-e (▲), and pepHda-n (△).

assays, variants of the pentameric consensus motif required more peptide (IC_{50} values of 70−80 μM) to inhibit DNA synthesis. PepHda-e was the most potent inhibitor. Interestingly, the peptides containing the native flanking sequences appeared to be better inhibitors than their DnaE-flanked counterparts, with IC_{50} values of 15 and 2.2 μM for pepMucB-n and pepHda-n, respectively (Table 2).

The data allow determination of the molar ratio of peptide required to inhibit the interaction of β_2 with the holoenzyme subunits (α and δ) in this assay system. For 50% inhibition of DNA replication, 1900 mol of the consensus-1 peptide is required for each mol of β_2, whereas 180 mol of pepHda-n or 1200 mol of pepMucB-n are required for the same extent of inhibition. Experiments were also carried out that showed that increasing the amount of β_2 in the reaction mixture reduced the extent of inhibition by the peptides (data not shown). This confirms that the binding of peptides to β_2 is responsible for inhibition of the DNA replication reactions.

Molecular Models of Peptide-β Complexes. Models for the structures of a penta- (QLSLF) and hexapeptide (QLSLPL) bound to monomeric β subunit were derived as described in the Materials and Methods and are depicted in Figure 6. The interactions between the peptides and β deduced from these models are shown in the Supporting Information (Figure S5). X-Score version 1.1 (*39*) computes the dissociation constants using an empirical scoring function calibrated with a set of 200 protein−ligand complexes and has an average accuracy of 1.5 pK_D units. This program predicts pK_D values of 6.24 (K_D = 0.58 μM) for the pentameric and 5.46 (3.74 μM) for the hexameric peptide models. These are comparable within the limits of accuracy of the method and are in reasonable qualitative agreement with the experimental data.

The majority of the interactions predicted for the pentamer QLSLF with β are through the Gln at position 1, Leu at position 4, and Phe at position 5. The model of the hexameric sequence, QLSLPL, can be compared to the structure of the β-binding sequence of Pol IV (DinB), QLVLGL (*26*, *27*). It is clear that the Pro can adopt a conformation that allows the two leucines to act as the "hydrophobic plug" in a manner similar to the LF of the δ subunit and pentamer β-binding sequences.

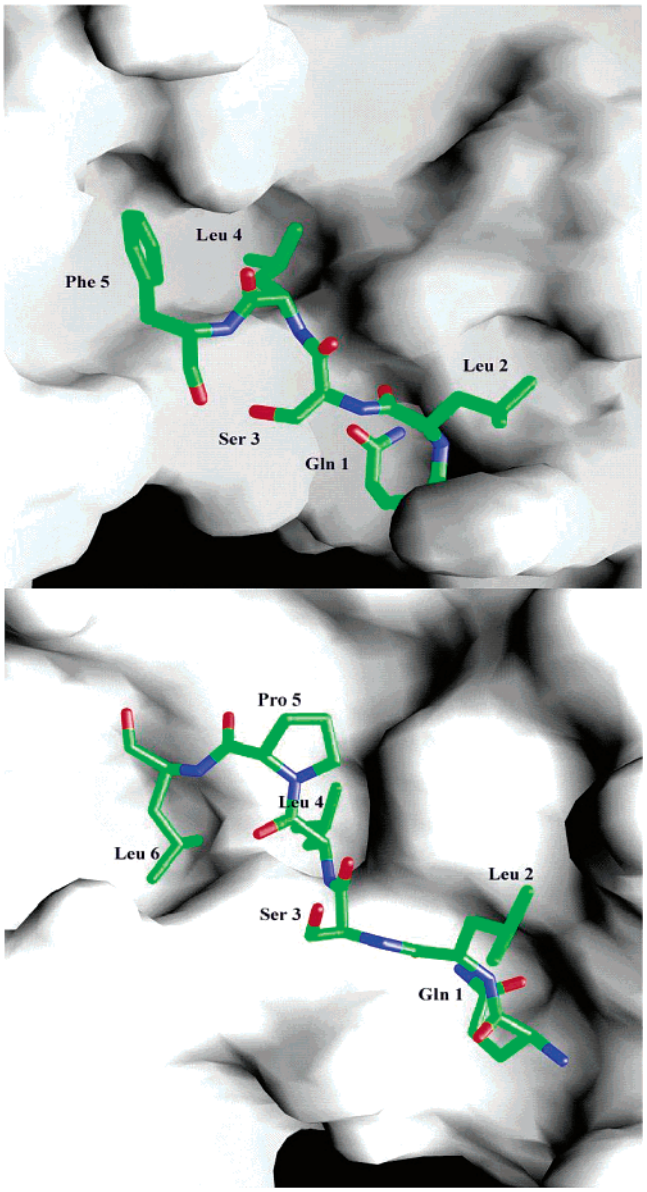

FIGURE 6: Computationally derived models for the β complexes with the consensus-2 pentameric peptide (A) and the hexameric β-binding peptide derived from Hda (B). Models were constructed as described in the Materials and Methods. Coordinates for the models are available from the authors, on request. The figure was prepared with GRASP version 1.3.6 (*42*).

DISCUSSION

This paper augments a previous report in which we identified a consensus pentameric sequence motif, QL(D/S)LF, in structurally and functionally distinct families of β_2-binding proteins from different species of eubacteria. The motif was derived from the most frequently observed amino acid at each of the five positions in the putative β_2-binding regions of the proteins (*24*, *28*). While we had previously shown that peptides containing the consensus sequences

inhibited $\alpha-\beta_2$ and $\delta-\beta_2$ interactions in simple plate assays and in yeast two-hybrid experiments (*24*), we wanted to determine the relative β_2-binding affinities of these consensus sequences and variants of them that occur naturally in the various families of *E. coli* β_2-binding proteins. Such a ranking should contribute to an understanding of the competition among proteins for binding to β_2 in its functions as a processivity factor for many polymerases.

Further bioinformatic analysis also led to the identification of a second motif, a hexameric sequence found in members of some polymerase families and Hda (*22*). Peptides with these sequences are for the first time examined here for β_2-binding activity and are compared to peptides containing the consensus pentapeptide sequences. In addition, the δ subunit is now known from crystallographic studies (*35*) to interact with β through a "hydrophobic plug" consisting of the Met, Leu, and Phe residues within the sequence QAMSLF. (This sequence also bears resemblance to the pentamer consensus sequence.)

Four series of peptides were investigated to compare the pentameric consensus sequences with naturally occurring variants and with putative hexameric β_2-binding sites: (i) a "DnaE series" of nonapeptides that included a putative α subunit (of Pol III) β_2-binding peptide, QADMF, and variants of it (*24*); (ii) several nonapeptides containing the consensus sequences, QL(D/S)LF, and variants of it; (iii) decapeptides containing the hexameric sequences; and (iv) peptides containing the hexameric β_2-binding peptide from the δ subunit, QAMSLF. Interference by peptides in β_2 binding to the α and δ subunits of Pol III holoenzyme was examined in several assay systems.

Three of these assays were designed to measure *inhibition* of functions of β_2 by peptides, including binding of β_2 to the α and δ subunits in plate assays, binding of β_2 to the immobilized consensus-1 peptide as assessed by SPR measurements, and β_2 functioning as the sliding clamp within the Pol III holoenzyme complex in a reconstituted in vitro DNA replication reaction. We did not expect that the actual values of IC_{50} (concentration of the peptide producing 50% inhibition) determined in the different assays with any particular peptide (or derivative) would be identical nor should it be a direct measure of the equilibrium dissociation constant of the β_2-peptide complexes. This is because the various assays measure β_2 function in quite different ways. For example, values obtained with the simple inhibition assays are expected to be sensitive to the concentration of competitor protein (α or δ), and the DNA replication assay measures the effect of peptides on the much more complex sequence of interactions of β_2 with δ and then α in the context of a reconstituted replisome. It was therefore most satisfying that the results indicated a consistent hierarchy of potency of the peptides, which would be expected if their complexation with β_2 interfered in a similar way with its interaction with both α and δ.

Within the series of pentameric sequences, it was shown that the consensus sequences were the strongest inhibitors and that there was some tolerance for substitutions at position 3 of the pentamer; this is the most variable site within the sequence (*24*, *28*). Substitutions at positions 4 and 5 of the consensus sequence resulted in lesser inhibitory activity. Although this is a limited sample, the data are consistent with extensive further studies with other sequence variants (G. Wijffels, J. Buchardt, and P. F. Alewood, unpublished data). This is also consistent with our constructed model of the complex between the consensus-2 pentameric peptide QLSLF and β (Figure 6A), where the Leu at position 4 and Phe at position 5 make extensive interactions with the hydrophobic pockets of β into which they insert, unlike the largely solvent exposed Ser at position 3.

The putative β_2-binding hexameric sequence from Hda was as good a competitor in these assays as the consensus pentameric sequence and might even be a slightly better inhibitor. The sequence from DinB1 showed less capacity as an inhibitor than the Hda sequence but was as good as the pentameric sequence from MutS. An alternative or second motif, also a hexameric peptide from the carboxyl-terminal tail of the α subunit (*40*), had not been reported at the time this work was done.

The other assay involved the direct measurement of thermodynamic parameters for binding of β_2 to immobilized peptides by SPR. While pepDnaE-n gave the highest values of K_D and the Hda sequences (in either orientation with respect to the surface) produced the lowest values, the data for the peptides containing the consensus sequence and variants were not entirely consistent with the results of the inhibition assays. This may reflect effects of biotinylation and/or immobilization of the peptides or the lack of ability of this assay to discriminate between similar molecules with such high dissociation rates and K_D values.

Peptides containing the β_2-binding region from the δ subunit (QAMSLF) were relatively poor inhibitors in the $\alpha-\beta_2$- and $\delta-\beta_2$-binding assays, despite the fact that the δ subunit and β_2 form a complex with a K_D of 7−10 nM, as measured by SPR (*11*). We note, however, that, although residues Met71, Leu73, and Phe74 (in this sequence) constitute 44% of the surface area buried on formation of the $\beta-\delta$ complex, they are not the only contacts that δ has with β_2. Jeruzalmi et al. (*35*) found additional contacts in their structure, including Gln69 (contributes an additional 12%). These extra interactions may be required for the peculiar function of the δ subunit in opening the β_2 ring after binding. Note that Gln69 of δ is probably not equivalent in position to the conserved Gln at position 1 of the penta- and hexapeptide motif (Figure 6) and that it is not a conserved residue across the family of δ subunits. The favorable electrostatic attractions at the salt bridge between Glu48 (and also Glu49) of δ and Arg152 of β and the likely hydrogen bond between His105 of δ and Arg365 of β probably contribute to the strength of their interaction.

Among the peptides examined here, the pentameric consensus sequence(s) QL(D/S)LF and the Hda hexameric sequence, QLSLPL, were the most potent inhibitors of interactions with β_2. Curiously, the replicative polymerase (α subunit of Pol III) has a poor β_2-binding motif. This is consistent with the lesser stability of the complex formed from α and β_2 in solution (cf. $\delta-\beta_2$), $K_D \sim 250$ nM, as determined by gel filtration (*4*). A peptide representing a second potential β-binding region on the α subunit was not tested in our assays. This peptide has been observed to displace the Pol III core complex from β_2 and inhibit DNA replication in vitro (*40*). It is interesting that this second sequence is not conserved among bacterial species outside the proteobacteriaceae. The two putative β-binding motifs of the α subunit are separated by a DNA binding domain,

and it is known that the α−β_2 complex is far more stable when bound at a replicative primer terminus, with a K_D value of ~5 nM (*23*). It is an intriguing possibility that two different sites in α interact simultaneously with the two equivalent sites in β_2, while the intervening region interacts with the double-stranded DNA passing through the center of the β_2 ring. Quantitative estimates of the stability of the complexes of β_2 with other intact polymerases and β_2-binding proteins have not yet been reported.

How these peptides associate with β_2 is not known. Clearly, there are clues from the δ−β crystal structure (*35*), and the SLF moiety of this β-binding region is conserved among sequences of the δ subunits. Moreover, in the structures of the Pol IV fragments complexed with β_2, two of the leucines make most of the intermolecular contacts (*26*, *27*). Nevertheless, positions 1 and 2 of the pentamer sequence appear to be very important for binding to β_2 (*24*; G. Wijffels and J. Buchardt, unpublished data). In our structural models, the Gln at position 1 of the pentamer interacts with Tyr323, Met362, Met364, and Arg365 of β, while the same Gln in the hexamer contacts Tyr323, Met362, and Asn320. Similarly, Gln364 of Pol IV makes contacts to Tyr323, Met362, Met364, and Asn320 of β_2 (*26*, *27*), and while Gln69 of the δ subunit makes contact with β (*31*), it is not conserved. This suggests that there might be subtle differences in the manner of binding by the various families of β_2-binding proteins. It is already known that when δ interacts with β_2, there are substantial structural rearrangements in both proteins (*31*) because this interaction is required to open the β_2 ring. On the other hand, as judged by the DinB−β_2 complexes, the interactions of β_2 with the polymerases might not involve such large conformational changes. It is reasonable to expect that the interactions of the peptides would produce fewer conformational changes in β_2. The small peptides may also make much more intimate contacts with β_2 than they would as part of a protein. While monomeric β is itself highly charged (total formal charge of −11 at neutral pH) and has large patches of negative electrostatic potential on its surface, the putative binding site of the peptides is hydrophobic and quite shallow. As in our models (Figure 6), the binding affinity is probably largely due to van der Waals interactions.

CONCLUSION

In this paper, we have examined the activities of several synthetic peptides containing penta- and hexameric motifs implicated in the binding of the β_2 sliding clamp protein to several polymerases (and other proteins) of the prokaryotic DNA replication and repair machinery. Despite fast kinetics, we found that peptides containing the pentameric consensus sequence and the δ and Hda hexameric sequences were capable of binding β_2 at concentrations in the high nanomolar range and inhibiting DNA synthesis in vitro and α−β_2 and δ−β_2 interactions at low micromolar concentrations.

The data suggest that the affinities of intact proteins in binding β_2 might vary in a hierarchical way. For example, the Hda hexapeptide seems to bind with higher affinity than that from δ or any of the pentapeptides, and interactions of pentapeptides from polymerases involved in translesion synthesis (TLS) are generally stronger than that with the natural sequence from the α subunit of Pol III. It is tempting to speculate that this order of binding affinity reflects events in DNA replication and repair in vivo: e.g., (i) the requirement to ensure tight control by Hda over reinitiation at *oriC*; (ii) the ease with which δ in the clamp loader needs to be able to pass β_2 to α in the replicase; and (iii) the need for facile exchange of β_2 from the replicase to a TLS polymerase when the replisome stalls at a site of DNA damage. Of course, the real situation is unlikely to be this simple; events in the replisome involve many other protein−protein and protein−DNA interactions that are intricately regulated by a series of switches, and it is very likely that some, at least of the β_2 interacting partners, have further interactions with β_2 outside their common peptide-binding site (*40*).

Our demonstration of inhibition of DNA synthesis is the first fully functional test of the capacity of these peptides to interfere with function of the chromosomal replicase. Further studies of inhibition of other polymerases in in vitro assays of their function would be informative, especially because the putative β_2-binding sites of Pol II, Pol IV, and Pol V are known to be functionally significant in β_2 binding and translesion DNA synthesis (*14*, *25*−*27*), and a 16-mer peptide from Pol IV has recently been shown to inhibit β_2-dependent DNA synthesis by that enzyme (*27*).

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SUPPORTING INFORMATION AVAILABLE

A total of 13 pages, including 5 figures, containing (i) details of the methods used for overexpression of α and for purification of α, δ, and δ′, (ii) SPR data showing the equilibrium β_2-binding isotherms generated by pepPolB-e, pepHda-e, pepMuC-n, pepDnaE-1, AcpepNH$_2$pepHda-n, and consensus-2, and (iii) LIGPLOT outputs mapping the interactions of β with consensus-2 peptide (QLSLF) and the hexameric β_2-binding sequence from Hda (QLSLPL). This material is available free of charge via the Internet at http://pubs.acs.org.

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