The complement system consists of about 30 proteins that play critical roles in both innate and adaptive immunity. Three different activation pathways initiate the complement response (1): the classical pathway (CP), the alternative pathway (AP), and the lectin pathway. Although each responds to different activating targets, which marks them for lysis and/or immune clearance (1) and primes them for the production of the high affinity antibody (2–4). C3a is an anaphylactic agent that focuses inflammatory reactions around foreign substances by inducing local vasodilation, the influx of leukocytes, the up-regulation of surface receptors, and the release of inflammatory mediators (5).

There are two structurally different C3 convertases (1): 1) The classical and lectin pathway convertase, C4b2a, traditionally termed the CP convertase and 2) the AP C3 convertase, C3bBb. Each is formed first through the association of C4b or C3b with a zymogen (C2 or factor B) in the presence of Mg\(^{2+}\). C2 and factor B are homologous proteins composed of three amino-terminal globular domains (complement control protein domains (CCPs)) followed by a CCP2/CCP3 site of DAF or structurally homologous sites of CR1 and C4BP.

Converting activity is controlled by the regulators of complement activation (RCA) proteins (6, 7) that irreversibly dissociate (decay accelerate) the convertase subunits or alternatively serve as cofactors for the proteolytic cleavage of C3b and C4b. Among the RCA proteins that mediate convertase dissociation, decay-accelerating factor (DAF), complement receptor 1 (CR1), and C4-binding protein (C4BP) dissociate the CP convertase, whereas DAF, CR1, and factor H dissociate the AP convertase. The purpose of this study was to provide information relevant to the molecular mechanisms that underlie decay accelerating activity.

To better understand decay accelerating activity, it is necessary to define specific regulator-convertase interactions. In a previous investigation we mapped sites in the type A domain of Bb that are involved in decay acceleration of the AP C3 convertase (C3bBb) (8). Like Bb, C2a is composed of an amino-terminal von Willibrand factor type A domain followed by a carboxyl-terminal serine protease domain (9). Several mutations in Bb centered at the adjacent \(\alpha\) helices 4 and 5, especially substitutions of Tyr\(^{388}\), caused C3bBb to exhibit marked resist-
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ance to DAF and CR1, and others at the a1 helix and its adjacent loops, especially D254G in the C3b-binding region of Bb, caused substantial resistance to DAF, CR1, and factor H. We hypothesized that the a4/5 region of Bb interacts directly with DAF and CR1, whereas the a1 helix and its adjacent loops affect the stability and dynamics of the Bb/C3b interface.

The RCA proteins are composed of tandem globular domains termed complement control protein repeats or short consensus repeats (6, 7). Only a portion of each protein is necessary for decay acceleration. Of the four CCPs of DAF, CCPs 2–4 are required for the decay acceleration of C3bBb, whereas only CCPs 2 and 3 are necessary to mediate decay of C4b2a (9, 10). In both cases about 10 amino acids, located in DAF CCPs 2 and 3, and the inter-CCP segment, appear critical (11). For CR1, CCPs 1–3 (of a total of 30 CCPs) are required for decay acceleration of both C3bBb and C4b2a (12), with a critical site in CCPs 1 and 2 structurally similar to that in DAF CCP2 and 3. For C4BP, CCPs 1–3 (of 8 CCPs) are required for decay acceleration of C4b2a (13).

Based on our previous mutagenesis work with DAF (8, 11), we hypothesized that the a4/5 region of Bb interacts with amino acids of DAF in CCP2 and CCP3. However, the ability of DAF to accelerate the decay of C3bBb also involves certain residues in its CCP4, so other models were feasible. Therefore, in the present study we focused on the decay acceleration of C4b2a, where only CCP2 and CCP3 are involved (9, 10). We examined the type A domain of C2a, identified C2a residues homologous to those Bb residues critical for decay acceleration of C3bBb, replaced the corresponding C2 amino acids by site-directed mutagenesis, and determined the effect of these mutations on the decay acceleration of C4b2a by DAF, CR1, and C4BP.

We found that the a4/5 region of C2a is critical for decay acceleration mediated by DAF, C4BP, and CR1, findings that strongly support the possibility that the a4/5 region of both C2 and factor B interact with the same elements of DAF CCP2 and CCP3. These new observations and our previous studies support the hypothesis that in decay acceleration of both C4b2a and C3bBb, the convertase a4/5 region interacts with a CCP2/CCP3 site of DAF or the structurally homologous sites of CR1 or C4BP. The results also indicate that C2 Tyr338 and homologous factor B Tyr338 are functional “hot spots” in the convertase/decay acceleration reaction.

EXPERIMENTAL PROCEDURES

Production of Mutant C2 Proteins—The plasmid containing wild type human C2 cDNA, pcDNA3C2SacR (14) originally from Dr. John Volanakis (University of California at Irvine) was obtained for the C241A positive control (19.6 ± 2.1 min) were comparable with native C2 protein (Advanced Research Technologies) and corresponded well to previous values obtained for this same mutant (15.7 min, 15)).

Protein Modeling—C2 (20) and factor B (21) amino acid sequences were aligned using the program SIM (Expassy) (22). The C2 wild type coordinates were constructed using InsightII software (Accelrys, San Diego, CA) using the primary sequence alignment shown in Ref. 25. The factor B type A domain model was previously constructed in a similar fashion (8). The C2a alignments of the type A domain model, with the HOMOLOGY module (Accelrys). In the experiments depicted in Fig. 6, 5 models were constructed for the wild type, and each Tyr252 mutant C2 protein and amino acids within a 2.5-Angstrom radius of position 327 were determined for each model.
A: CCP 1-2-3- Linker – vWF Type A Domain – Linker – Serine Protease Domain

B: 230

f B

GEGGKQRKVLDFSGSNYTVLDGDCSDDGASNTFTGKKSCLVNLIEKAVYGVKFRGTVY

C2

TKESLRGKRQIQRESSGHNLVLDDDCSQVSENDFLIFKESASLMVDRIFSFEEINVSVAILTF

219

310

f B

TYPKIVWVEASDANWNWKLQINEYDHLKSKTNTKLADAVYSSMSMPD---EVPPE

C2

SEPKULSVDNSPTEVISSHNLLENKHDGNTITNYALNSVTLTNNQMLRGMEN

299

342

360

f B

GNWRTHIVIIIMTDGGLNMDGPDITVEIDEIOLLYGKDRKPNREDVLVYFGVYGLVNO

C2

AWQEIRHAILLDGKSMMGSPPATRTHIREILNQKNR---DILDIYAIAGVGLDVMRR

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Fig. 1. Alignment of the factor B and C2 type A primary sequences. A, sequential domains of factor B and C2. B, primary sequences of von Willbrand factor type A domain and subsequent linker. Bold and underlined letters indicate the locations of substituted C2 residues and factor B homolog. Asp240 and Ser244 are two of the five Mg2+ coordination residues; mutation of these residues resulted in loss of C2 activity. Gln243, Lys254, and Glu255 are in the 1 helix; Asn324, Tyr327, and Leu328 are in the 4 helix; and Asp434 is in the 7 helix (linker). The arrows indicate substituted residues with greatest negative impact on decay accelerating activity.

RESULTS

Construction of a Panel of C2 Type A Mutants—As indicated (in the Introduction): the a4/5 region, especially the Tyr327 residue, and a1 and its adjoining a1/2 loop, especially the Asp323 residue, were identified as important for decay acceleration of C3bBb. To determine whether corresponding regions are important in the decay acceleration of C4b2a, we aligned the primary sequences of the factor B and C2 type A domain and connecting linker (Fig. 1). We found conservation of the Tyr338 homolog, i.e. C2 residue Tyr327, and although the analog of factor B Asp240 is glutamine (C2 residue Gln243), we found that its position remains within a conserved group of five putative Mg2+ coordination residues (23).

Based on the above alignment, we constructed a panel of eight C2 type A domain mutants (Table I) corresponding to mutations in factor B previously shown to affect decay acceleration (8). The recombinant proteins were transiently expressed in human 293T kidney cells and were quantitated as described in the Experimental Procedures. Each protein was examined by Western blotting, assayed for its sensitivity to C1s cleavage, and analyzed for its hemolytic capacity. All of the mutants, except as noted below, assembled similarly to wild type C2 and retained hemolytic activity. Their activities require functioning C4b-binding sites in both the C2a and C2b regions, a Mg2+ coordination site located at the type A domain of C2a, an intact C1s cleavage site located at the junction between C2b and C2a, and a working C3/C5 substrate recognition site as well as a functional serine protease catalytic site in the serine protease domain of C2a (Fig. 1). The fact that these sites are distributed throughout C2 strongly argues that the mutations made in each case induced only relatively local structural changes.

Effects of C2 Mutations on Decay Acceleration—C4b2a was assembled with wild type or mutant C2, and the resulting convertases were compared for their sensitivity to DAF, CR1-A, and C4BP (Figs. 2 and 3). Consistent with our previous studies on C3bBb (8), the a4 mutation Y327A dramatically reduced the sensitivity of C4b2a to DAF- and CR1-A-mediated decay acceleration to less than 1% of that of wild type C4b2a. Additionally as found with C3bBb, alanine substitutions near Tyr327 (N324A and L328A) also reduced decay acceleration but to a lesser degree. The Q243G substitution, located in the divalent cation-binding cleft (23) corresponding to the putative C4b-binding region (25), likewise markedly reduced C4b2a sensitivity to DAF (2% of wild type) and moderately reduced C4b2a sensitivity to CR1-A (55% of wild type). Decay acceleration of C4b2a mediated by C4BP was affected by the C2 mutations in much the same way as that by DAF and CR1-A (Fig. 3).

To determine whether the Y327A and Q243G mutations affected intrinsic convertase function, we examined convertase assembly and spontaneous decay. As seen in Fig. 4, both mutant proteins were readily cleaved by C1s. Assembly of C4b2a with Y327A occurred at nearly wild type rate, whereas Q243G enhanced the assembly rate (data not shown). When compared with wild type C2 (19.6 ± 2.1 min), the Y327A mutation had little if any effect on C4b2a half-life (14.9 ± 4.9 min). Interestingly, Q243G increased C4b2a half-life (to 51.5 ± 15.5 min), a result similar to that obtained with the factor B analog D254G (26). Thus, in the case of the CP C3 convertase, Y327A inhibited regulator-mediated dissociation but not spontaneous dissociation, whereas Q243G inhibited both regulator-mediated and spontaneous dissociation.

In contrast to findings for factor B, in which the conservative Y338F mutation reduced DAF sensitivity to 18% and CR1-A sensitivity to 20% (8), the homologous C2 conversion, Y327F, had no detectable effects on DAF sensitivity and only a very modest effect on CR1-A sensitivity (67% of wild type). Also contrary to their factor B counterparts (Table I and Discussion), neither the a1 mutation (K254A, E255A) nor the a7 mutation (D434A) had detectable effects on decay acceleration by any of the regulators.

Topology of the C2 and Factor B a4/5 Regions—To compare the a4/5 structures of the two proteins, we performed homology modeling (24) and constructed three-dimensional models of the wild type C2 and factor B type A domains using as a reference the coordinates of the type A domain of CR3 derived by x-ray crystallography (23). As seen in Fig. 5, analysis of these models indicated that C2 Tyr327 and factor B Tyr338 are each part of a cluster of polar and charged amino acids that reside on the a4 and a5 helices. As noted above, mutation of residue Asn324, part of the putative C2 cluster, as well as Leu328, adjacent to
The C2 and factor B type A models are approximations based on the crystallographic structure of the homologous type A domain from the α subunit of CR3 (23). We reasoned that examination of a number of type A models of each key mutation might help clarify why Tyr327 is critical to decay acceleration.

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In 74 of the 75 models, the position 327 side chain was in van der Waals contact with at least one α5 side chain, although the specific α5 contact depended on the specific position 327 residue (Fig. 6A). Contact between position 327 and α5 residue Ile378 occurred in 92% of the wild type and Y327F models but only 44% of the Y327A models, and contact between position 327 and α5 residue Glu374 occurred in 40% and 24% of the wild type and Y327F models but only 4% of the Y327A models (Fig. 6, B and C). Differences in position 327 contacts within the α4 helix were also observed. Contact between position 327 and α4 residue Asn324 was seen in 48% of the wild type models and 56% of the Y327F models but in none of the Y327A models. In summary, when compared with wild type Tyr327 by these modeling methods, the Y327F replacement permits fewer side chain contacts both between α4 and α5 helices and within the α4 helix, whereas the conservative Y327F replacement permits an array of side chain contacts similar to the wild type Tyr327 models.

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In 74 of the 75 models, the position 327 side chain was in van der Waals contact with at least one α5 side chain, although the precise α5 contact depended on the specific position 327 residue (Fig. 6A). Contact between position 327 and α5 residue Ile378 occurred in 92% of the wild type and Y327F models but only 44% of the Y327A models, and contact between position 327 and α5 residue Glu374 occurred in 40% and 24% of the wild type and Y327F models but only 4% of the Y327A models (Fig. 6, B and C). Differences in position 327 contacts within the α4 helix were also observed. Contact between position 327 and α4 residue Asn324 was seen in 48% of the wild type models and 56% of the Y327F models but in none of the Y327A models. In summary, when compared with wild type Tyr327 by these modeling methods, the Y327F replacement permits fewer side chain contacts both between α4 and α5 helices and within the α4 helix, whereas the conservative Y327F replacement permits an array of side chain contacts similar to the wild type Tyr327 models.

**Table 1**

| C2 mutation     | Predicted location | Factor B homolog | C3bBb sensitivity to DAF | C3bBb sensitivity to CR1-A |
|-----------------|--------------------|------------------|--------------------------|---------------------------|
| Q243G           | α1/2 loop          | D254G            | 9                        | 1                         |
| K254A/E255A     | a1                 | K285A/K266A      | 18                       | 18                        |
| N324A           | α4                 | O335A            | 20                       | 22                        |
| Y327A           | α4                 | Y338A            | 3                        | 1                         |
| N324A/Y327A     | α4                 | ND               | ND                       | ND                        |
| Y327F           | α4                 | Y338F            | 18                       | 20                        |
| L328A           | α4                 | S339A            | 32                       | 50                        |
| D434A           | α7                 | D445A            | NS                       | 71                        |

*The values are the percentages of wild type.

**Fig. 2.** DAF-mediated decay acceleration of C4b2a. C4b2a was assembled on the surface of sheep erythrocytes (EshA) using mutant or wild type C2, and the cells were incubated with control buffer or with DAF at various concentrations for 15 min and then subjected to lysis via the complement terminal pathway (see “Experimental Procedures”). Decay acceleration (Inhibition of hemolysis) was calculated as the percentage in the Z value (the average number of lytic sites per erythrocyte) caused by incubation with DAF. In this experiment, the decay accelerating activity of the wild type recombinant C2 protein was compared with that of the two C2 type A mutants, Y327A and N324A.

**Fig. 3.** The effects of C2 mutations on decay acceleration of C4b2a. In the case of C4b2a assembled with wild type C2, decay acceleration (inhibition of hemolysis) caused by the activity of regulator, was defined as 100%. The effects of each mutation on sensitivity to decay acceleration were compared with the wild type (set) for each regulator. Regulator concentrations were: CR1-A, 172.7 ng/ml; C4BP, 700 ng/ml; DAF, 6 ng/ml. Approximate molar equivalents based on molecular masses of 69, 550, and 27.8 kDa, respectively, were 2.50, 1.27, and 0.22. At these regulator concentrations, hemolysis of wild type convertase was inhibited by 40–50%.

**Fig. 4.** C1s-mediated cleavage of C2 mutants. C2 forms (indicated above the lanes) were incubated with buffer alone in the odd-numbered lanes and with C1s in the even-numbered lanes. A, Y327A substitution compared with native wild type C2. B, Q243G substitution compared with wild type recombinant C2.

**DISCUSSION**

The C3 convertases, the central amplification enzymes of the complement cascade, initiate several potent inflammatory processes (see the Introduction). Their strict regulation is essential to permit the elimination of foreign agents while at the same time protecting self-tissues from autologous complement-mediated injury. The RCA family of regulators has evolved to serve this purpose. The mechanism by which these proteins control the activity of the convertases is by accelerating their irreversible dissociation (decay acceleration). As indicated (in the In-
Decay Acceleration Sites of the CP C3 Convertase

We previously showed that for factor B, mutations in two sites, the parallel \( \alpha_4 \) and \( \alpha_5 \) helices and the \( \alpha_1 \) helix and its adjacent A2 loop, are important in DAF- and CR1-mediated decay acceleration of C3bBb (8). In the current study we found that homologous regions of C2 are similarly important in DAF- and CR1-mediated as well as C4BP-mediated decay acceleration of C4b2a.

One major finding was that alanine substitution of the C2 type A residue Tyr327 results in a complex that is highly resistant to the decay acceleration activity of DAF, CR1, and C4BP, and alanine substitution of the neighboring C2 residues Asn324 and Leu328 renders the complex partially resistant (Fig. 3). These mutants otherwise functioned normally, as did their factor B homologs (8). The results strongly support the proposition that the \( \alpha_4/5 \) region of the CP C3 convertase interacts with all three regulators. Taken together with our previous finding that the homologous factor B Tyr338 residue is involved in both DAF and CR1-mediated decay acceleration, the results highlight a central importance for this site in the decay acceleration of both the CP and AP C3 convertases.

In general, protein-protein interfaces have been described as large bodies of relatively weak interactions that are punctuated by a few “hot spots” (27), amino acids whose interactions are responsible for most of the binding energy. Hot spot residues are relatively conserved and often polar (28, 29). The residues surrounding hot spots are usually less conserved and have a more subtle influence on binding (27–29), possibly serving to exclude bulk solvent and retard the attack of water molecules on the interface core (27, 28). The \( \alpha_4/5 \) regions of C2 and factor B each define such a cluster of polar/charged surface residues (Fig. 5) that could be part of the structural and functional core of a convertase-regulator interface.

Previously we showed that substitution of Tyr338 with Phe reduced the sensitivity of C3bBb to both DAF and CR1-A, but substitution of Tyr338 with Ala resulted in a much greater loss, indicating that both the 4-hydroxyl group and the phenyl group
are important (8). In this study, the C2 Y327F substitution had less effect on CR1-A sensitivity of C4b2a and no detectable effect on DAF or C4BP sensitivity, indicating that the role of the phenyl group is more critical than that of the 4-hydroxyl group. Modeling studies provided evidence that the phenyl group (but not the hydroxyl group) plays a direct role in the contacts between the α4 and α5 helices and within the α4 helix (see under “Results” and Fig. 6 (A and B)). Although the Tyr327 phenyl group could be a contact point for regulator interaction, the modeling studies suggest two additional nonexclusive mechanisms to account for the insensitivity of Y327A to decay acceleration: 1) the Y327A substitution alters the relative orientation of the α4 and α5 helices, thus changing the relative positions of regulator contact points in α4/5 (such as N324A and L328A), and 2) the Y327A substitution interferes with the dynamics between the α4 and α5 helices, which could be critical to convertase-regulator interaction (via an “induced fit” model) or to the propagation of allosteric changes promoted by the convertase-regulator interaction.

The interaction of C4b2a with DAF may afford the simplest model for decay acceleration because as indicated (see the Introduction) only two DAF CCPs, CCP2 and CCP3 (9, 10), are involved, whereas decay acceleration by all other RCA proteins appears to involve at least three CCPs (12, 13, 30). Moreover, unlike the other regulators, DAF does not have cofactor activity for the factor I-mediated cleavage of C4b or C3b. Although the affinity of DAF for C4b2a and C3bBb appears very low (indeed it has never been demonstrated directly), indirect evidence suggests a role in the stabilization of the C4b2a convertase since DAF and C4BP, and to a lesser extent, CR1-A. These substitutions also increase convertase stability in the absence of regulator (this study and Ref. 8). Unlike Y327A and Q243G, two other C2 substitutions, K254A/E255A in α1 and D434A in α7, did not affect decay acceleration in our experiments. In the case of the homolog D445A in factor B, a small but statistically significant difference in the sensitivity to CR1-A (71% wild type) was found (8). In the case of the factor B homolog K265A/K266A, the differences were greater (about 18% sensitivity to DAF, CR1-A, and factor H) and may be attributable to effects on the C3b-Bb interface (8). Parallel effects were not detected with our K254A/E255A C2 mutants, the simplest interpretation being structural differences between C4b and C3b in their respective convertases.

In summary, our findings indicate that the α4/5 region of C2a is critical to decay acceleration mediated by DAF, C4BP, and CR1. Importantly, the results indicate that C2 Tyr237 and homologous factor B Tyr328 constitute a functional “hot spot” in the convertase/decay accelerator reaction. The available evidence suggests two simple interpretations: 1) that decay acceleration of C4b2a and C3bBb requires interaction of the convertase α4/5 region with the mapped CCP2 and CCP3 sites of DAF or structurally homologous sites of CR1 and C4BP and 2) that decay acceleration requires the propagation of an allosteric signal from regulator-convertase interface to the C4b2a (C3bBb) interface, which involves participation of the α4/5 region. In either model, the region plays an important role in a common mechanism to destabilize C4b2a and C3bBb.

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REFERENCES

1. Volanakis, J. E. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds) 10th Ed., pp. 9–32, Marcel Dekker, Inc., New York
2. Pearson, D. T., and Locksley, R. M. (1996) Science 272, 50–54
3. Carroll, M. C., and Prodeus, A. P. (1998) Curr. Opin. Immunol. 10, 36–40
4. Nielsen, C. H., Fischer, E. M., and Leslie, R. G. (2000) Immunology 106, 4–12
5. Englund, F., and Hugli, T. E. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds) 10th Ed., pp. 211–244, Marcel Dekker, Inc., New York
6. Liszewski, M. K., and Atkinson, J. P. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds) pp. 149–166, Marcel Dekker, Inc., New York
7. Ahearn, J. M., and Rosengard, A. M. (1998) in The Human Complement System in Health and Disease (Volanakis, J. E., and Frank, M. M., eds) pp. 167–202, Marcel Dekker, Inc., New York
8. Hourcade, D. E., Mitchell, L., Kuttner-Kondo, L. A., Atkinson, J. P., and Medof, M. E. (2000) J. Biol. Chem. 275, 1107–1112
9. Brodbeck, W. G., Liu, D., Sperry, J., Mold, C., and Medof, M. E. (1996) J. Immunol. 156, 2528–2533
10. Kuttner-Kondo, L. A., Lin, F., Hourcade, D. E., and Medof, M. E. (2002) Int. Immunopharmacol. 2, 1275
11. Kuttner-Kondo, L. A., Mitchell, L., Hourcade, D. E., and Medof, M. E. (2001) J. Immunol. 167, 2164–2171
12. Krych-Goldberg, M., Hauhart, R. E., Subramanian, V. B., Yurecsik, B. M., II, Crimmins, D. L., Hourcade, D. E., and Atkinson, J. P. (1999) J. Biol. Chem. 274, 31160–31168
13. Blom, A. M., Kask, L., and Dahlback, B. (2001) J. Biol. Chem. 276, 27136–27144
14. Tsukamoto, H., Tousson, A., Marchase, R. B., and Volanakis, J. E. (1996) J. Immunol. 156, 4991–4998
15. Horiuchi, T., Macon, K. J., England, J. A., and Volanakis, J. E. (1991) J. Immunol. 147, 584–589
16. Hourcade, D. E., Wagner, L. M., and Oglesby, T. J. (1995) J. Biol. Chem. 270, 19716–19722
17. Oglesby, T. J., Accavitti, M., and Volanakis, J. E. (1988) J. Immunol. 141, 2559–2561
18. Brodbeck, W. G., Kuttner-Kondo, L., Mold, C., and Medof, M. E. (2000) Immunology 101, 104–111
19. Krych, M., Clemenza, L., Howdeshell, D., Hauhart, R., Hourcade, D., and Atkinson, J. P. (1994) J. Biol. Chem. 269, 12773–12778
20. Bentley, D. R. (1986) Biochem. J. 230, 339–345
21. Mole, J. E., Anderson, J. K., Davison, E. A., and Woods, D. E. (1984) J. Biol. Chem. 259, 5407–5412
22. Huang, Q. G., and Miller, W. (1991) Adv. Appl. Mathematics 12, 337–357
23. Lee, J. O., Rieu, P., Arnaout, M. A., and Ledingham, R. (1995) Cell 80, 631–638
24. Stossel, T. P., and Blundell, T. L., Adv. Immunol. 234, 779–815
25. Hinselwood, J., Spencer, D. I. R., Edwards, Y. J. K., and Perkins, S. J. (1999) J. Mol. Biol. 294, 578–599
26. Hourcade, D. E., Mitchell, L. M., and Oglesby, T. J. (1999) J. Immunol. 162, 2906–2911
27. Clackson, T., Uttech, M. H., Wells, J. A., and de Vos, A. M. (1998) J. Mol. Biol. 277, 1111–1128
28. Bogus, A. A., and Thorn, K. S. (1998) J. Mol. Biol. 280, 1–9
29. Hu, Z., Ma, B., Wolfson, H., and Nussinov, R. (2000) Proteins 39, 331–342
30. Kuhn, S., and Zipfel, P. F. (1996) Eur. J. Immunol. 26, 2383–2387
31. Pangburn, M. K. (1986) Adv. Protein Chem. 342–357
32. Kuttner-Kondo, L., Medof, M. E., Brodbeck, W., and Shoham, M. (1996) Protein Eng. 9, 1143–1149
33. Kollscha, T., Medof, M. E., and Nussenzweig, V. (1986) J. Immunol. 136, 3390–3395
34. Uehara, S., Lin, F., Ball, G., Bromek, K., Uhrin, D., Medof, M. E., and Barlow, P. N. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4718–4723
35. Williams, P., Chaudary, Y., Goodfellow, I. G., Billington, J., Powell, R., Spiller, O. B., Evans, D. J., and Lea, S. (2003) J. Biol. Chem. 278, 10691–10696