Demonstration of Different Metal Ion-induced Calcineurin Conformations Using a Monoclonal Antibody*

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It has been suggested that calcineurin, a calmodulin-stimulated phosphatase, may exist in different metal ion-dependent conformational states (Pallen, C. J., and Wang, J. H. (1984) J. Biol. Chem. 259, 6134-6141). Evidence in favor of this hypothesis comes from studies involving a monoclonal antibody, VA1, which is specific for the small (β) subunit of calcineurin. This antibody inhibits Ni²⁺-stimulated but not Mn²⁺-stimulated phosphatase activity against p-nitrophenyl phosphate and phosphorylase kinase. Inhibition is not due to competition of the antibody with substrate or to interference with metal ion binding to the enzyme. Complex formation between the antibody and calcineurin can be demonstrated either in the presence of Mn²⁺ or Ni²⁺ or in the absence of metal ion activators. These results indicate that the active conformational states of calcineurin are metal ion dependent, that the monoclonal antibody VA1 affects the Ni²⁺-induced conformational change of the enzyme, and that the β subunit of calcineurin plays a critical role in the expression of Ni²⁺-stimulated phosphatase activity.

The major calmodulin-binding protein in bovine brain, calcineurin, is composed of two subunits: α, M₀ = 61,000 and β, M₀ = 20,000 (1-3). This protein has been demonstrated to possess a metal ion-dependent and calmodulin-stimulated protein phosphatase activity (4). Although initially thought to possess a relatively narrow substrate specificity, calcineurin has recently been shown to dephosphorylate several phosphoseryl, phosphothreonyl, and phosphoaspartyl-containing proteins, p-nitrophenyl phosphate and free phosphotyrosine, and phosphoenolpyruvate (4-9). It is, however, not firmly established that the multiple phosphatase activities are due to a single enzyme species.

Using the chromogenic substrate p-nitrophenyl phosphate, calcineurin-phosphatase activity has been shown to depend on metal ion activators such as Mn²⁺ or Ni²⁺. Different metal ion-activated calcineurin-phosphatases appear to have different catalytic properties. These enzyme forms are not readily interconvertible. These results have led to the suggestion that calcineurin may exist in different metal ion-dependent conformations (10).

Recently several monoclonal antibodies to calcineurin have been developed and characterized in our laboratories. One of these is specific toward the β subunit of the protein whereas others are α subunit specific. In this study we show that the β subunit-specific monoclonal antibody VA1 is capable of inhibiting Ni²⁺-activated calcineurin activity but not Mn²⁺-activated enzyme activity. This inhibition by the antibody can be demonstrated when either protein or nonprotein substrates are used. The results suggest that multiple phosphatase activities are intrinsic properties of calcineurin and substantiate the suggestion that calcineurin can exist in different metal ion-dependent conformational states.

MATERIALS AND METHODS

Proteins—Calcineurin and calmodulin were purified from bovine brain as previously described (11). Phosphorylase kinase was purified from rabbit skeletal muscle according to published procedures (12). The monoclonal antibody VA1 was developed and purified from mouse ascites fluid using a DEAE-Affi-Gel blue column (13).

Assay for Calcineurin-Phosphatase Activity—Calcineurin activity against pNPP was assayed as described previously (10). Briefly, calcineurin was preincubated with either 1 mM metal ion as indicated or 1 mM EDTA at 25 °C for an hour in the presence of BSA (0.5 mg/ml). Calcineurin activity was assayed in 0.5 ml of a reaction mixture containing 50 mM Tris-HCl (pH 7.2), 1 mM metal ion, 2.7 mM pNPP, 0.5 mg/ml BSA, and in the presence or absence of 1.5 μM calcineurin at 30 °C. The reaction was started by the addition of 50 μl of 13% K₂HPO₄ and read at the wavelength 405 nm, or the time course of the reaction was followed on a Bausch and Lomb 2000 spectrophotometer.

NaDODSO₄-Polyacrylamide Gel Electrophoresis and Immunoblotting—Siab gel electrophoresis in the presence of 0.1% NaDODSO₄ was carried out according to the procedure of Lasmi (14) using 7.5-15% acrylamide gradients. Protein bands were visualized using the silver staining method as described by Wray et al. (15).

Immunoblotting was carried out essentially as described by Towbin et al. (16). The electrophoretic transfer of protein was carried out in the presence of 0.1% NaDODSO₄, to increase the transfer efficiency. The detection of the cross-reactive protein band was carried out using alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin G ( Sigma). The blots were developed on an ultraviolet transilluminator using 4-methylumbelliferyl phosphate as a substrate (17).

High Performance Liquid Chromatography—The HPLC gel filtration column TSK-3000 SW (7.5 x 300 mm) from LKB was used. Elution buffer contained 20 mM Tris acetate and 0.1 M NaH₂PO₄ (pH 7.2). The sample was prefiltred using HPLC nylon filters (5-mm pore size).

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2 The abbreviations used are: DEAE, diethylaminoethyl; HPLC, high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; NaDODSO₄, sodium dodecyl sulfate; EDTA, ethylene-diaminetetraacetic acid; pNPP, p-nitrophenyl phosphate; Tris, tris(hydroxymethyl)aminomethane; BSA, bovine serum albumin.
diameter, 0.45-μm pore size). Elution was done at room temperature and at a flow rate of 0.33 ml/min. The column eluate was monitored for UV absorbance at 280 nm and was fractionated every minute (0.33 ml/fraction). The fractionated samples were analyzed by Na-DodSO₄-polyacrylamide gel electrophoresis and silver staining.

RESULTS

Further Characterization of the Monoclonal Antibody \( \text{VA}_1 \)-
Five monoclonal antibodies have been purified and characterized in our laboratories. Only one of these antibodies, \( \text{VA}_1 \), appears to be specific for the \( \beta \) subunit of calcineurin, as determined by immunoblotting. Immunoblotting analysis of \( \text{VA}_1 \) reactivity toward purified bovine brain calcineurin and crude extract of rat brain is shown in Fig. 1. In both cases a polypeptide with a molecular weight identical to \( \beta \) subunit strongly reacts with the antibody, while \( \alpha \) subunit does not react at all. A protein band, present in both purified calcineurin and in crude rat brain extract, with a molecular weight lower than \( \beta \) subunit also reacts with \( \text{VA}_1 \). In addition, several polypeptides with molecular weights higher than that of \( \alpha \) subunit showed consistent but weak reactivity with \( \text{VA}_1 \). These results indicate that \( \text{VA}_1 \) is specific for the \( \beta \) subunit of bovine and rat brain calcineurin but may possess weak cross-reactivity with several minor components of brain, some of which appear to be present in the purified calcineurin sample. Since the cell line producing the monoclonal antibody \( \text{VA}_1 \) was subcloned three times, these reactions are unlikely to be due to contamination of \( \text{VA}_1 \) with a different antibody clone. The nature of these minor components is unclear, but they may be higher molecular weight nondissociable forms of calcineurin (for example \( \alpha \beta_2 \)) or, in the case of the smallest reactive polypeptide, a degradation product of the \( \beta \) subunit.

Effect of \( \text{VA}_1 \) on Calcineurin-Phosphatase Activity—The ability of the various monoclonal antibodies to inhibit \( p \)-nitrophenyl phosphatase activity of calcineurin was tested. Only \( \text{VA}_1 \) was found to have an inhibitory effect, and this effect is dependent upon the metal ion used to activate the enzyme. Previously we have shown that \( p \)-nitrophenyl phosphatase activity of calcineurin requires a metal ion activator, a requirement which may be satisfied by either Mn\(^{2+}\) or Ni\(^{2+}\). Fig. 2 shows that \( \text{VA}_1 \) markedly inhibits phosphatase activity of Ni\(^{2+}\)-stimulated calcineurin but only marginally affects the activity of Mn\(^{2+}\)-stimulated calcineurin.

The concentration dependence of \( \text{VA}_1 \) inhibition of Ni\(^{2+}\)-stimulated calcineurin activity (Fig. 2) suggests that inhibition results from a stoichiometric interaction of 1 molecule of calcineurin with 1 molecule of antibody. At a molar ratio of antibody to enzyme of 1:1 about 75% inhibition of activity is observed (Fig. 2). Increasing antibody concentration up to a molar ratio of 4:1 resulted in only a small additional extent of inhibition. At the highest antibody concentration used, 20% of the original enzyme activity remained, indicating that the fully saturated immunoenzyme complex possesses residual catalytic activity.

Inhibition of Ni\(^{2+}\)-activated \( p \)-nitrophenyl phosphatase activity by \( \text{VA}_1 \) is not competitive with respect to substrate (Fig. 3), indicating that inhibition does not arise from the binding of the antibody to the active site of the enzyme. Both \( V_{\text{max}} \) and \( K_m \) of the dephosphorylation reaction are altered following immunoenzyme complex formation. The suggestion that the antigenic site is distinct from the active site on the enzyme is also compatible with the observation that the inhibition of enzyme activity is incomplete even at high antibody concentration.

The possibility that the antibody inhibits calcineurin-phosphatase by interfering with Ni\(^{2+}\) binding to the enzyme or by causing dissociation of Ni\(^{2+}\) from the enzyme has been con-

![Fig. 1. Immunoblotting analysis of the monoclonal antibody \( \text{VA}_1 \). Purified bovine brain calcineurin (1 μg) and rat brain crude extract (105,000 × g supernatant, 350 μg) were electrophoresed on Na-DodSO₄-PAGE. Proteins were electrophoretically transblotted onto nitrocellulose filters as described under “Materials and Methods.” The nitrocellulose blots were incubated with 3% BSA in Tris saline for 1 h at 40 °C and then incubated with 27 pg/ml monoclonal antibody in Tris saline with 3% BSA for 2 h. The blot was then washed with Tris saline and incubated with alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin G (Sigma). The blot was developed as described under “Materials and Methods.” As a control the same samples were processed without incubation with the monoclonal antibody \( \text{VA}_1 \) solution. In this case no protein bands could be seen. Arrows show the position of each subunit (\( \alpha \) and \( \beta \)) of calcineurin on the nitrocellulose. A, purified bovine brain calcineurin; B, rat brain crude extract.](image1)

![Fig. 2. Effect of \( \text{VA}_1 \) monoclonal antibody on calcineurin-phosphatase activity toward \( p \)-nitrophenyl phosphate. Calcineurin (25 μg/ml) was preincubated with 2.5 mM Ni\(^{2+}\) or Mn\(^{2+}\), and the preincubated calcineurin (2.5 μg) was further incubated with different amounts of \( \text{VA}_1 \) antibody for 30 min. The phosphatase activity was assayed in the presence of calsomulin (10 μg), 1 mM Ni\(^{2+}\) or Mn\(^{2+}\), and \( p \)-nitrophenyl phosphate (1 mg/ml), and with Ni\(^{2+}\)-activated (○) or Mn\(^{2+}\)-activated (●) calcineurin.](image2)
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Calcineurin was preincubated with 1 mM Ni²⁺. Preincubated calcineurin (1.5 µg) was further incubated with different amounts of VAI monoclonal antibody for 30 min. Calcineurin-phosphatase activity was assayed with 1 mM Ni²⁺, 10 pg of calmodulin, and different concentrations of pNPP as a substrate, either without antibody (○) or with 1.2 µg (●) or 1.8 µg (△) of VAI.

Effect of VAI on Phosphophosphorylase Kinase Phosphatase Activity of Calcineurin—In addition to p-nitrophenyl phosphatase activity of calcineurin, phosphoenolpyruvate phosphatase activity is also inhibited by VAI antibody in a metal ion-dependent manner (8). To test if phosphoprotein phosphatase activity of calcineurin was inhibited in a like way, the dephosphorylation of phosphorylase kinase was examined. Fig. 6 shows that both Ni²⁺ and Mn²⁺ support calcineurin-phosphatase activity toward phosphorylase kinase. In this case, the Mn²⁺-stimulated calcineurin-phosphatase activity is slightly higher than that of the Ni²⁺-stimulated enzyme, while Ni²⁺-stimulated p-nitrophenyl phosphatase activity is higher than Mn²⁺-stimulated enzyme activity. Similar to reactions involving nonprotein substrates, VAI only inhibits Ni²⁺-stimulated activity toward phosphorylase kinase.

Demonstration of Calcineurin and VAI Interaction by HPLC Gel Filtration—In view of the specific inhibition of Ni²⁺-activated calcineurin activity by VAI, it is possible that the antibody binds only to the Ni²⁺-induced enzyme conformation and not to the Mn²⁺-induced form of calcineurin. Alternatively, the antibody, although complexing with all forms of calcineurin, may only affect the activity of the Ni²⁺-induced calcineurin conformation. To distinguish between these possibilities, direct interaction between various forms of calcineurin and VAI monoclonal antibody was examined using

FIG. 3. Lineweaver-Burk plot of the inhibition of calcineurin-phosphatase by VAI antibody. Calcineurin was preincubated with 1 mM Ni²⁺. Preincubated calcineurin (1.5 µg) was further incubated with different amounts of VAI monoclonal antibody for 30 min. Calcineurin-phosphatase activity was assayed with 1 mM Ni²⁺, 10 pg of calmodulin, and different concentrations of pNPP as a substrate, either without antibody (○) or with 1.2 µg (●) or 1.8 µg (△) of VAI.

FIG. 4. Effect of different concentrations of Ni²⁺ on the inhibitory activity of VAI. Calcineurin (0.2 mg/ml) was preincubated with 1 mM Ni²⁺. Preincubated calcineurin (10 µg) was then incubated with 15 µg of VAI antibody (—) or without antibody (— - -). Phosphatase activity was monitored spectrophotometrically in the absence of calmodulin. The Ni²⁺ concentration was serially increased. The assay was started with 0.1 mM Ni²⁺ and then increased to 0.3, 0.5, 1, and 2 mM Ni²⁺ at the times indicated by the arrows.

FIG. 5. Effect of VAI on calmodulin stimulation of phosphatase activity. A, calcineurin (0.2 mg/ml) was preincubated with 1 mM Ni²⁺. The Ni²⁺-activated calcineurin (1.5 µg) was further incubated with (●) or without (△) 10 µg of VAI for 30 min. Calcineurin activity was assayed with 1 mM Ni²⁺ and with different amounts of calmodulin. B, calcineurin (0.2 mg/ml) was preincubated with 1 mM Ni²⁺. The Ni²⁺-activated calcineurin (1.5 µg) was then incubated with different amounts of VAI antibody for 30 min. Phosphatase activity was measured with 1 mM Ni²⁺ and in the presence or absence of 15 µg of calmodulin. Calcineurin activity in the absence of calmodulin was taken as 100% activity.
Incubation Time (min)

**FIG. 6.** Effect of VA1 monoclonal antibody on Ni2+- or Mn2+-stimulated calcineurin activity toward phosphorylase kinase. Phosphorylase kinase was autophosphorylated in the presence of [γ-32P]ATP (specific activity, 193 cpm/pmol). Three and a half mol of phosphate were incorporated per mol of phosphorylase kinase. Calcineurin was preincubated with 1 mM Ni2+ (A) or Mn2+ (B) in the presence of BSA (0.5 mg/ml). Reaction mixture containing 0.5 pg of activated calcineurin and 2 mM Ni2+ (A) or Mn2+ (B) was incubated either with 1 pg of VA1 (○) or with the equivalent amount of buffer (○) for 30 min. Controls contain no calcineurin (○). Dephosphorylation experiments were started by the addition of phosphophosphorylase kinase (19.8 pg). At the times shown above, aliquots of the reaction mixture were removed, and the reaction was stopped with 5% trichloroacetic acid, and radioactivity in the supernatant counted.

HPLC gel filtration. Fig. 7 shows the gel filtration profiles of calcineurin and of VA1 alone and of calcineurin and VA1 antibody preincubated in the presence of EDTA, Ni2+, or Mn2+. Purified calcineurin was resolved in a single protein peak (Fig 7A), while the VA1 filtration profile showed one major protein peak and a minor shoulder peak, the latter of which represents transferrin contamination of VA1 (Fig. 7B). On this gel filtration column calcineurin and VA1 eluted at very similar positions despite their different molecular weights. Four poorly resolved peaks were seen when calcineurin and VA1 mixtures were preincubated with EDTA or Ni2+ and chromatographed (Fig. 7, C and D). The elution times of the second, third, and fourth peaks correspond to those of VA1, calcineurin, and transferrin, respectively. The first peak, corresponding to a component with a higher molecular weight than any single protein applied to the column, contains both calcineurin and VA1, as shown by NaDodSO4-PAGE and represents the enzyme-antibody complex. When calcineurin and VA1 were preincubated with Mn2+ and then chromatographed (Fig. 7E), the first peak seen in Fig. 7, C and D, was reduced to a shoulder of a larger second peak. Although the first peak was not as pronounced in this case, NaDodSO4-PAGE revealed the presence of both calcineurin and VA1, indicating the occurrence of complex formation. A preincubated mixture of purified nonimmunized mouse immunoglobulin G and calcineurin was chromatographed as a control for the specificity of the calcineurin-VA1 interaction (Fig. 7F). Two very poorly resolved peaks are apparent while no peak corresponding to an antibody-enzyme complex is seen.

**DISCUSSION**

Five hybridoma cell lines producing specific calcineurin antibodies have been produced in our laboratories. We have investigated the usefulness of these antibodies as probes to investigate the structural and functional properties of calcineurin. The studies described here exclusively involved the monoclonal antibody VA1, since it is the only antibody which...
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that to a contaminant in the protein preparation. The observation that VA inhibits the activity of calcineurin toward certain protein and nonprotein substrates indicates that these multiple activities reside in the same protein species.

Marked inhibition of calcineurin-phosphatase activity was observed only when calcineurin was activated by Ni\(^{2+}\), while Mn\(^{2+}\)-activated calcineurin activity was only marginally affected. The inhibition by the antibody of Ni\(^{2+}\)-activated phosphatase activity is incomplete since significant activity is still observed at a saturating antibody level. Kinetic analysis indicates that inhibition of Ni\(^{2+}\)-activated calcineurin activity by the monoclonal antibody does not appear to be due to the binding of the antibody to the active site of the enzyme; this is not surprising since the antibody is directed against the \(\beta\) subunit of calcineurin while the catalytic site is reported to reside on the \(\alpha\) subunit of the enzyme (18). The antibody also does not affect Ni\(^{2+}\) binding to calcineurin. Since both Ni\(^{2+}\) and Mn\(^{2+}\) appear to bind to the same site(s) on calcineurin (10), the differential inhibition of these metal ion-stimulated enzyme activities by the antibody does not seem to be related to the interaction of antibody with the metal binding site(s) on the enzyme. The antibody VA complexes with Ni\(^{2+}\)-activated, Mn\(^{2+}\)-activated, and metal-free calcineurin, demonstrating that the absence of enzyme inhibition observed with Mn\(^{2+}\)-activated calcineurin is not due to a lack of enzyme-antibody interaction.

As well as inhibiting Ni\(^{2+}\)-stimulated enzyme activity, VA antibody also reduces the calmodulin-stimulated activity of calcineurin. This may be due to one of the following possibilities: (i) it is possible that the antibody decreases the affinity of calcineurin for calmodulin; (ii) that calmodulin binding to calcineurin is blocked by antibody; or (iii) that the enzyme affinity for calmodulin binding is unaffected but that the calmodulin-induced conformational change of calcineurin is inhibited by the antibody. The observation that increasing amounts of calmodulin do not overcome the reduction in calmodulin-stimulated enzyme activity makes the first possibility seem unlikely. The second possibility is also unlikely, since some calmodulin stimulation, although reduced, is observed even in the presence of saturating levels of antibody and since the antibody binds to the \(\beta\) subunit of calcineurin, while the calmodulin-binding site is located on the \(\alpha\) subunit (1).

In a previous study (10) we have suggested that calcineurin may exist in various metal ion- and Ca\(^{2+}\)-calmodulin-dependent forms, based on observations that the mechanism and rates of calcineurin activation by Ni\(^{2+}\) and Mn\(^{2+}\) are different and that Ni\(^{2+}\)- and Mn\(^{2+}\)-activated forms of calcineurin are not readily interconvertible except via an interaction with Ca\(^{2+}\)/calmodulin. The differential inhibition of Ni\(^{2+}\)- and Mn\(^{2+}\)-activated calcineurin by the monoclonal antibody further supports such a suggestion. Our results indicate that the \(\beta\) subunit is essential for Ni\(^{2+}\) stimulation but not Mn\(^{2+}\) stimulation of calcineurin activity. It is also possible that the \(\beta\) subunit plays a role in both Ni\(^{2+}\)- and Mn\(^{2+}\)-stimulated enzyme activity and that the antibody is able to prevent the former but not the latter process. In either case, inhibition may only be due to interference with the ability of the \(\beta\) subunit to modulate enzyme activity, suggesting that the conformational effects of antibody binding are confined to the \(\beta\) subunit of calcineurin. However, the antibody-induced inhibition of Ni\(^{2+}\)-stimulated calcineurin activity also results in a reduction of the calmodulin stimulation of enzyme activity. The location of the calmodulin-binding domain on the \(\alpha\) subunit of calcineurin and our above discussion of the likelihood that the reduction in calmodulin-stimulated enzyme activity is due to an inhibition of the calmodulin-induced conformation change of calcineurin suggest that antibody binding to the \(\beta\) subunit effects a conformational change in the \(\beta\) subunit of the enzyme which is conferred to the \(\alpha\) subunit. Since both the calmodulin-dependent and -independent Ni\(^{2+}\)-stimulated but not Mn\(^{2+}\)-stimulated enzyme activities are inhibited by the antibody, we would like to propose that Mn\(^{2+}\)- and Ni\(^{2+}\)-treated calcineurins possess different active conformations of which the Ni\(^{2+}\)-induced conformation is prevented or reversed by VA, while the Mn\(^{2+}\)-induced conformational change occurs or is not reversed even after antibody complexation.

Since the inhibition of phosphatase activity is not due to the binding of VA, at the active site of calcineurin, our results do not indicate that the \(\beta\) polypeptide is the catalytic subunit of the enzyme. It is interesting to note that all other monoclonal antibodies produced against the \(\alpha\) subunit do not inhibit calcineurin activity (see Footnote 1 and Ref. 18). The results of this study suggest that \(\beta\) subunit plays a critical role in the Ni\(^{2+}\) activation of calcineurin-phosphatase. The combined use of this monoclonal antibody and physical techniques may reveal the nature of the involvement of the \(\beta\) subunit in the Ni\(^{2+}\)-induced conformational changes of calcineurin.

The application of monoclonal antibody to the elucidation of structure-function relationships and regulatory properties of proteins has been stressed by many investigators (for review, see Ref. 19). The present study is limited to answering a few questions which, in our opinion, are of fundamental importance to understanding the mode of action of calcineurin. The results demonstrate that both certain protein and nonprotein phosphatase activities are intrinsic to calcineurin, support the suggestion that calcineurin exists in various metal ion-dependent conformational states, and suggest that \(\beta\) subunit plays a role in the Ni\(^{2+}\)-induced conformational changes of the enzyme. Clearly, monoclonal antibodies may be used to investigate many other properties of calcineurin. For example, previous studies have suggested that calcineurin in cells or in crude tissue extracts may contain a tightly bound metal ion activator (10, 20). Monoclonal antibodies may be used to test such a possibility and to identify the physiological metal ion activator. In addition, monoclonal antibodies specific for the two different subunits of calcineurin may be used to test whether the two subunits have identical subcellular distributions under various physiological conditions.

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REFERENCES

1. Sharma, R. K., Desai, R., Waisman, D. M., and Wang, J. H. (1979) J. Biol. Chem. 254, 4276-4282
2. Klee, C. B., Crouch, T. H., and Kranks, M. H. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6270-6273
3. Aitken, A., Klee, C. B., and Cohen, P. (1984) Eur. J. Biochem. 139, 663-671
4. Stewart, A. A., Ingebritsen, T. S., Manalan, A., Klee, C. B., and Cohen, P. (1982) FEBS Lett. 137, 80-84
5. Yang, S. D., Taliant, E. A., and Cheung, W. Y. (1982) Biochem.
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6. King, M. M., and Huang, C. Y. (1983) *Biochem. Biophys. Res. Commun.* 114, 955–961
7. Pallen, C. J., and Wang, J. H. (1983) *J. Biol. Chem.* 258, 8550–8553
8. Wang, J. H., Pallen, C. J., Brown, M. L., and Mitchell, K. J. (1984) *Fed. Proc.* 43, 1897
9. Chernoff, J., Sells, M. A., and Li, H.-C. (1984) *Biochem. Biophys. Res. Commun.* 121, 141–148
10. Pallen, C. J., and Wang, J. H. (1984) *J. Biol. Chem.* 259, 6134–6141
11. Sharma, R. K., Taylor, W. A., and Wang, J. H. (1983) *Methods Enzymol.* 102, 210–219
12. Cohen, P. (1973) *Eur. J. Biochem.* 34, 1–14
13. Bruck, C., Portetelle, D., Glineur, C., and Bollen, A. (1982) *J. Immunol. Methods* 53, 313–319
14. Laemmli, U. K. (1970) *Nature (Lond.)* 227, 680–685
15. Wray, W., Boulikas, T., Wray, V. P., and Hancock, P. (1981) *Anal. Biochem.* 118, 197–203
16. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 4350–4354
17. Shalev, A., Greenberg, A. H., and McAlpine, P. J. (1980) *J. Immunol. Methods* 38, 125–139
18. Winkler, M. A., Merat, D. L., Tallant, E. A., Hawkins, S., and Cheung, W. Y. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 3054–3058
19. Benjamin, D. C., Berzofsky, J. A., East, I. J., Gurd, F. R. N., Hannum, C., Leach, S. J., Margoliash, E., Michael, J. G., Miller, A., Prager, E. M., Reichlin, M., Sercarz, E. E., Smith-Gill, S. J., Todd, P. E., and Wilson, A. C. (1984) *Annu. Rev. Immunol.* 2, 67–101
20. King, M. M., and Huang, C. Y. (1984) *J. Biol. Chem.* 259, 8847–8856