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Solution Structures of Calcium Regulating Proteins: A Small-Angle Scattering Study

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Small-angle X-ray scattering (SAXS) experiments have shown that the solution structures of two calcium-binding regulatory proteins, calmodulin and troponin C, are significantly different from their crystal structure forms. The structural differences occur in a region of calmodulin that is thought to bind to target enzymes; the calmodulin-enzyme complex is an initiator for many important biochemical processes. Calcium binding to calmodulin induces a conformational change that is a prerequisite for calmodulin binding to a target enzyme. SAXS data can help characterize this conformational change and give insight into the mechanism of enzyme binding. Neutron resonance scattering promises to determine accurately the distances between calcium binding sites, thus providing important constraints on the structure of calmodulin in solution.

INTRODUCTION

Proteins form a special class of polymers and are made up of combinations of 20 chemical groups referred to as amino acids. A given protein possesses a unique sequence of amino acid residues that are connected via covalent peptide bonds to form a chain (the alpha-carbon backbone) that is decorated by the reactive groups that characterize the amino acids (the side-chains). Although a given protein contains a wider variety of monomers than synthetic polymers, every molecule is made from the same template and hence samples are monodisperse. Furthermore, conformational energies are identical for all identical molecules, so they all assume the same shape(s) and low-resolution scattering is able to give somewhat more shape information than in the case of synthetic polymers.

The chemical and physical properties of the amino acid side-chains determine the 3-dimensional structure (and structural dynamics) of a given protein, and hence its biochemical function. Thus, proteins display an enormous variety of structural and functional diversity, playing crucial roles for example in enzyme catalysis, transport and storage of small molecules, coordinated motion, immune response, mechanical support, neurotransmission, and the control of growth and differentiation. The relationship between structure and function of proteins is one of the most intensely investigated areas in modern biochemistry and biophysics.

Regulation of biochemical processes is generally achieved via the interactions of small molecules with proteins. There are 3 major classes of small molecules that regulate biochemical processes. They are the hormones, cyclic nucleotides and the calcium ion (Ca^{2+}).[1] The calcium ion is the major regulator of intracellular processes that occur on short time scales ranging from milliseconds to seconds. Calcium regulation usually involves Ca^{2+} binding to a protein after some event that resulted in an influx of Ca^{2+} to a system. It is thought that the Ca^{2+}/protein complex undergoes a conformational change that allows it to combine with a target enzyme and trigger a biochemical reaction. A number of calcium binding protein structures have been solved by X-ray crystallography and they have been shown to display a high degree of sequence and structural homology.[2,3,4,5,6] The known structures have either two or four calcium binding sites per protein molecule and each of the sites displays a characteristic helix-loop-helix structural motif (Figure 1). The proteins that bind only two calcium ions are similar to other water soluble proteins, in that they show a globular structure that tends to minimize the surface area exposed to the polar environment, while buried hydrophobic interactions on the interior of the molecule contribute significantly to the structural stability of the molecule. The proteins that bind four calcium ions, on the other hand, have a very unusual tertiary structure.

Calmodulin and troponin C are proteins that each bind four calcium ions and their high-resolution crystal structures are known.[4,5,6] Calmodulin is a multifunc-

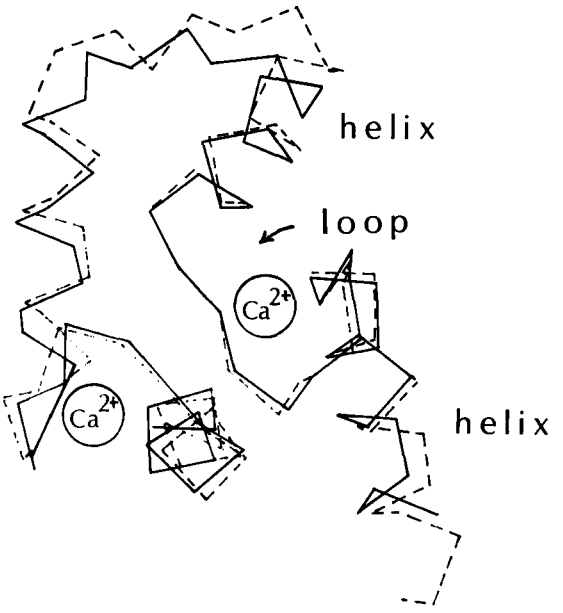

FIGURE 1 The alpha-carbon backbone of the globular domains for calmodulin showing the helix-loop-helix configuration of the calcium binding sites. The solid line represents the *C*-terminal domain while the dashed line represents the *N*-terminal domain. The domains have been overlayed to demonstrate their close structural homology.

tional calcium regulatory protein that controls a wide variety of biochemical processes,[7,8,1] while troponin C is responsible for regulation in muscle systems.[9] The structures of these proteins show two calcium-binding domains connected by an extended alpha helix that is exposed to solvent and forms few contacts with the rest of the molecule (see Figure 3a). The calcium-binding domains show a high degree of structural homology to each other (Figure 1) as well as to the proteins which bind only two calcium ions. The forces which stabilize the interconnecting helix in the crystal form, however, are not well understood. This is an important issue since a number of studies suggest that the interconnecting helix, as well as portions of each domain, form the target enzyme binding site (see reference 10 and references therein).

Small-angle scattering is a valuable technique for studying the overall shape of macromolecules in solution.[11,12] This paper describes small-angle scattering experiments aimed at determining the structures of calmodulin and troponin C in a physiological solution environment. For brevity, the troponin C results have been omitted, although the conclusions regarding its solution structure are qualitatively very similar to calmodulin.

Materials and Methods

X-ray scattering data were collected on a small-angle scattering station at Los Alamos. This instrument uses a sealed tube source and slit geometry. Data were analyzed by both the Guinier approximation and an indirect Fourier transform analysis. Samples for measurement were prepared from purified protein in buffers that approximated physiological conditions (150 mM ionic strength, pH 7.4). Studies were done to compare experimental results with those predicted from models based on the crystal structure of calmodulin. The modeling algorithm is based on a Monte Carlo integration technique which derives a $P(r)$ function, radius of gyration R_g, and maximum linear dimension d_{max} for a particular model. The sample preparation, X-ray scattering station, data analysis and modeling procedures are all described in detail elsewhere.[13]

Effects of Calcium Binding to Calmodulin

Figure 2 shows the $P(r)$ curves for calmodulin with and without calcium bound, while Table I shows the corresponding R_g and d_{max} values. These results are in good agreement with similar measurements done by Seaton and coworkers.[14] When calcium binds to calmodulin there is an increase in the number of vectors longer than 35 Å and a decrease in the number of shorter vectors. This results in increases in both d_{max} and R_g.

Calmodulin is able to bind and activate its target enzymes only in the presence of calcium. NMR data[10] has implicated the interconnecting helix region of calmodulin, as well as regions of both globular domains, as being part of the target enzyme binding site. We have shown that calcium binding results in an elongation of calmodulin. Spectroscopic data[15,16] demonstrates that there is an accompanying 4% increase in alpha-helix content. There is additional data[17,18,19] that indicates that calcium binding to calmodulin also results in the exposure of hydrophobic

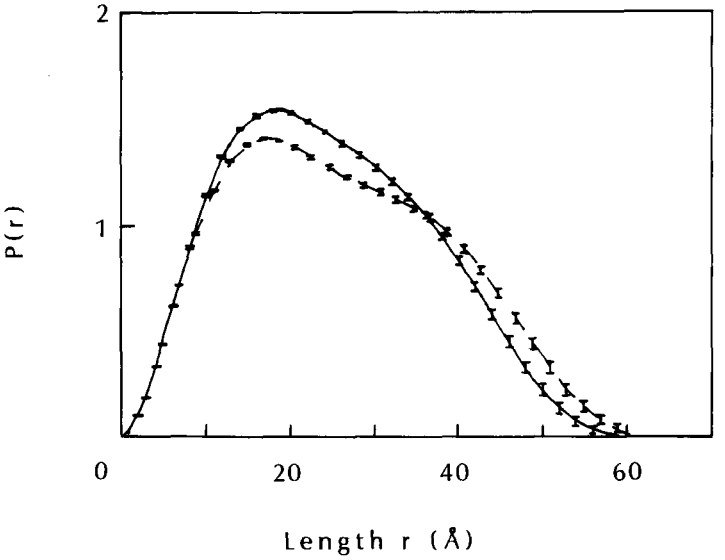

FIGURE 2 The experimental $P(r)$ functions for calmodulin in solution, with (---) and without (——) calcium bound. P(r) is the probable frequency distribution of vector lengths in the scattering particle (calmodulin).

regions of the molecule. These data suggest that activation of calmodulin involves a calcium-induced conformational change that results in a lengthening of the structure, a possible stabilization of part of the interconnecting region in a helical conformation, and the exposure of hydrophobic patches that may provide points of interaction with the target enzyme.

Comparison of the Crystal and Solution Structures of Calmodulin and Troponin C

Figure 3 shows the crystal structure alpha-carbon coordinates of calmodulin superimposed on a uniform density ellipsoid model that approximates the molecular boundary of the crystal structure. The $P(r)$ function obtained from this model does not agree well with the experimental curve (Figure 3c). The crystal structure $P(r)$ has two well resolved maxima at approximately 17 Å and 45 Å. These two maxima of the calculated $P(r)$ function are characteristic of a "dumbell" shaped molecule; the first maximum is near the value of the radius of each of the end domains and the second (less intense) maximum is near the value of the distance between the centers of the two end domains. The experimental curve shows the maximum at 17 Å, but only a weak shoulder at longer vectors (approx. 39 Å); that is, the second maximum has moved to shorter vector length and the valley between the two maxima is filled in, showing a higher probability of intermediate length vectors. These observations suggest that while the size and shape of the globular domains are similar in solution and in the crystal, the distance between them is smaller. The differences between the experimental and crystal structure $P(r)$ curves are well beyond the errors derived from the experimental data.[13] Further, the R_g and d_{max}

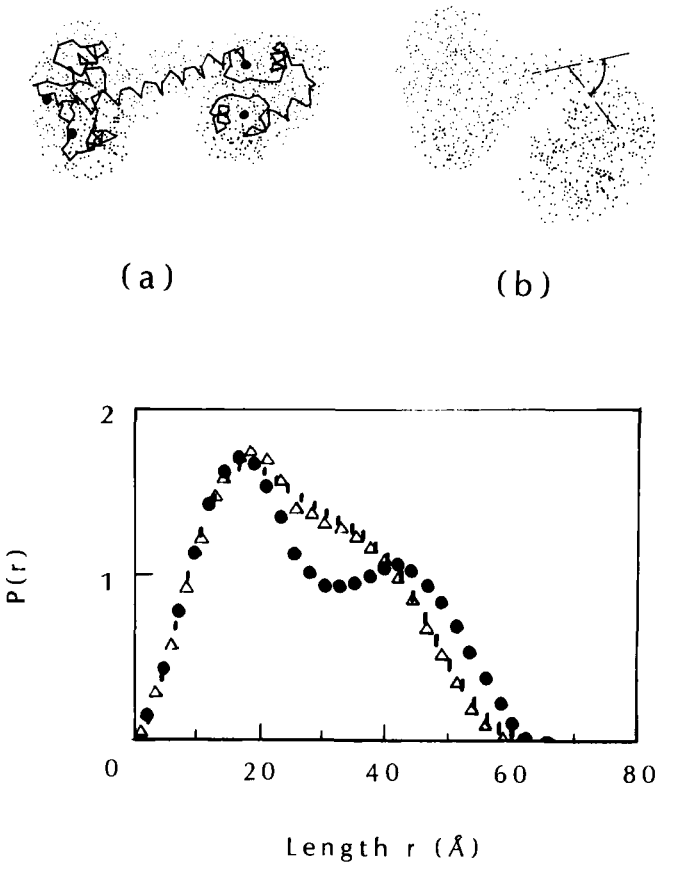

FIGURE 3 (a) The crystallographic alpha-carbon backbone superimposed on the dot representation of the crystal structure model, (b) the dot representation of the "bent" model and (c) the $P(r)$ functions calculated for calmodulin using the uniform ellipsoid model that best represents the crystal structure (●), the "bent" model (△), and the experimental curve (|). The size of the symbols represents the errors propagated from statistical errors in the data and corrections such as slit smearing.

values derived from the crystal structure are significantly larger than the experimental values from solution scattering (Table I).

Excellent agreement can be obtained with the experimental curve, however, by rearranging the electron density in the interconnecting helix region and rotating the globular domains so that they are brought closer to one another. Such a model is shown in Figure 3b, and its $P(r)$ in Figure 3c. In this model, the centers of mass of the two ellipsoids representing the globular domains are closer to one another by 5 Å, and the distance of closest approach of the ellipsoid surfaces is smaller by 9 Å compared to the model of the crystal structure. The R_g and d_{max} of the "bent" model also show excellent agreement with the experimental values (Table I).

TABLE I

R_g and d_{max} Values Determined for Calmodulin and Troponin C[a]

		R_g(Å)	d_{max}(Å)	
Calmodulin				
Ca^{2+}, pH 7.4	P(r)	21.3 ± 0.2 [21.5 ± 0.2]	63 ± 2 [62]	
	Guinier	21.0 ± 0.6		
EGTA[b], pH 7.4	P(r)	19.6 ± 0.1 [20.6 ± 0.2]	59 ± 2 [58]	
	Guinier	19.9 ± 0.4		
Crystal structure		22.8	70	
"Bent" model		21.2	62	
Troponin C				
Mg^{2+}, pH 7.4	P(r)	23.0 ± 0.2	70 ± 3	
	Guinier	23.0 ± 0.2		
Crystal structure		23.9	72	
"Bent" model		22.7	68	

[a]Values in square brackets are from Seaton et. al.[14]
[b]EGTA samples contained no Ca^{2+}

The crystal structure and experimental $P(r)$ curves for troponin C show similar discrepancies as are observed for calmodulin, and improved agreement with the experimental data is also obtained by moving the globular domains closer together[13] (data not shown). This is to be expected in view of the high degree of sequence and structural homology between the two proteins.

Solution scattering data are limited in resolution and can only give information about the overall shape of a molecule in solution. Further, because molecules in solution are free to reorient, the data are isotropically averaged and it is therefore not usually possible to prove one particular model correct on the basis of solution scattering data alone. It is possible, however, to test models. What is clear from these studies is that the crystal structures do not predict the solution scattering data, based on comparisons of R_g and d_{max} values, and particularly on comparisons of the shapes of the $P(r)$ functions. Including larger uniform hydration layers in the structures was found not to improve the agreement. Models have been derived that predict the solution scattering data very well, but other possibilities may exist. It is also possible that in solution there are a number of conformational substates and that the distances between the globular domains could vary over a range of values and the solution scattering reflects only the "average" conformation.

The "bent" structures, or population of structures, considered in analyzing the solution scattering results are attractive because they only require modifications to the crystal structure in the region that appears to be the most likely region of structural instability. The globular domains of calmodulin are similar to globular domains in other proteins and would be expected to be stable in solution. The interconnecting helix, however, has very few contacts with the rest of the molecule, and this has lead to speculation as to the stability of this region. Sundaralingham and coworkers[20] have proposed that salt bridges within the helical regions might be important in stabilizing the interconnecting helix in troponin C. Herzberg and James[5] have suggested that in troponin C the interconnecting helix may not be stable in solution and could fold around the "helix-breaker" glycine (at position

92 in the chain) that occurs in the helix region, thus they propose a mechanism by which the two globular domains could interact. Such effects will be critical to a complete understanding of the mechanism of action of calmodulin in solution.

Neutron Resonance Scattering as a Structural Probe for Calmodulin

The problem of spherical averaging in solution scattering, and hence lack of unique solutions, can be partly overcome if models can be constrainted by known parameters. For example, knowledge of the precise distance between the calcium binding sites in the calmodulin solution structure would significantly constrain the number of possible models for that structure. Many investigators have attempted to use spectroscopic techniques to determine these distances, but these measurements are plagued by large errors due to the size of labels used or assumptions concerning their dynamics.[21,22] We have therefore proposed a small-angle neutron scattering experiment in order to address this issue.

The experiment will utilize the novel technique of neutron resonance scattering from plutonium (^{240}Pu) ions which have been substituted for Ca^{2+} ions in the calcium binding sites of calmodulin. The neutron scattering cross section of ^{240}Pu at the resonance energy of 1.057 eV (or wavelength 0.278 Å) is approximately 1000 times that of other nuclei (Figure 4),[23] so that scattering from ^{240}Pu at 1.057 eV

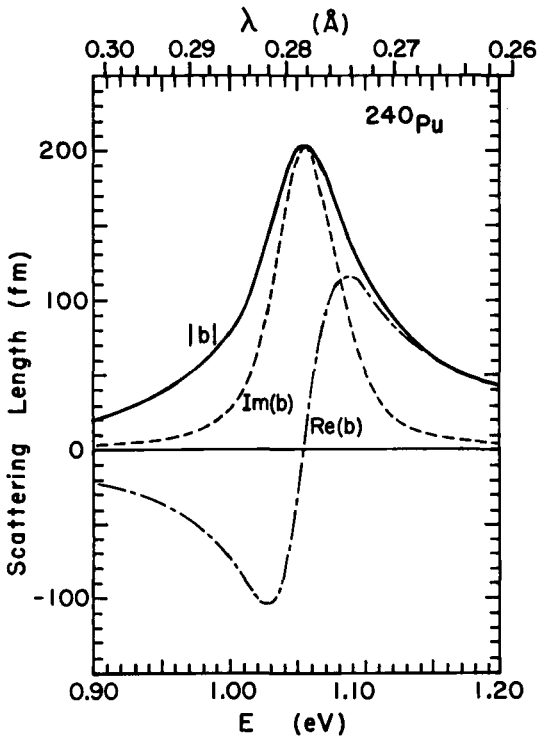

FIGURE 4 Coherent scattering length for ^{240}Pu in the vicinity of the resonance at 1.057 eV. The scattering length, b, is a complex quantity. The amplitude of b at the resonance wavelength is approximately 30 times that of other nuclei; scattering cross sections is $4\pi b^2$.

will predominate over scattering from other nuclei. In addition, the phase of the resonance scattering length is orthogonal to non-resonance scattering, so only ^{240}Pu–^{240}Pu correlations will give coherent scattering at the resonance energy. To obtain the scattering due to the ^{240}Pu ions alone, data acquired away from the ^{240}Pu resonance are subtracted from that acquired on resonance. Figure 5 shows an analytical calculation of the scattering cross section for 4 plutonium ions arranged approximately as in the crystal structure for calmodulin. The low-frequency oscillations in the pattern arise from the average intra-domain distances, while the high frequency oscillations arise from inter-domain distances.

The experiment described can be done on the Low-Q Diffractometer (LQD)[24] at the Los Alamos Neutron Scattering Center because the spallation source provides a white neutron spectrum extending to very short wavelengths. Particular neutron energies are selected by time of flight from source to detector. As a measure of the resolution of the LQD at 1.057 eV, a 1.6 mg/cm^2 sample of ^{240}Pu was placed in the beam, and the transmission was measured as a function of neutron energy. The shape of the resonance was clearly resolved in 30 minutes of running time. The sample for the proposed experiment will contain only one-tenth this number of ^{240}Pu atoms, and the scattering cross section (as shown per molecule in Figure 5) is only 9% of the absorbtion cross section. Therefore, much longer running times will be required.

Several questions arise concerning the binding of plutonium to calmodulin. First,

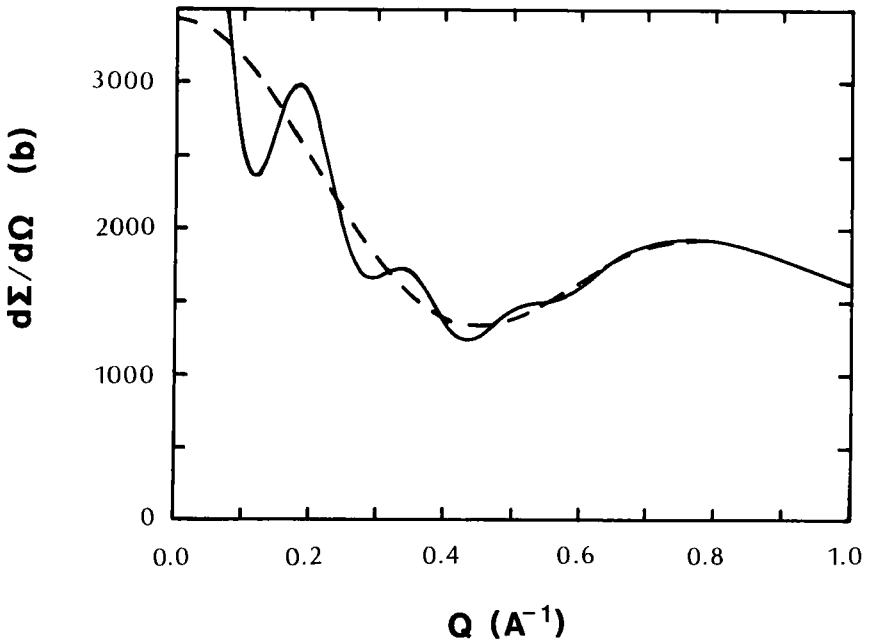

FIGURE 5 Computed scattering pattern for calmodulin with all Ca^{2+} binding sites occupied by ^{240}Pu, assuming a 10 Å spacing between two sites within a domain, and a Gaussian distribution of spacings between sites in separate domains with mean 40 Å and standard deviation 2.5 Å.

will there be significant non-specific binding? Second, will the conformational changes induced by ^{240}Pu binding be different from Ca^{2+}? The $^{240}Pu^{3+}$ ion has approximately the same size electron cloud as Ca^{2+} and it is expected to bind with high affinity to the calcium binding sites of calmodulin. Work is underway to characterize this binding (in collaboration with D. Hobart, G. Jarvinan, and S. Rokop). Small-angle X-ray scattering experiments similar to those described above will be able to test whether or not there have been large scale conformational rearrangements induced by plutonium binding. Spectroscopic techniques (e.g. circular dichroism) will be able to assess the effects of plutonium on secondary structure, and possibly also probe for small changes in the calcium binding pockets. If it can be shown that the structure of calmodulin is not dramatically perturbed by the plutonium binding, then the distances obtained from a ^{240}Pu resonance scattering experiment will provide important constraints for modeling calmodulin structure in solution, and the resonance neutron scattering technique can be applied to other similar systems, for example troponin C. In view of the differences observed between the high resolution crystal and solution structures for these proteins, such constraints will be crucial to our understanding of the mechanism of action of calmodulin in solution.

References

1. W. Y. Cheung, *Science 207*, 19 (1980).
2. R. H. Kretsinger and C. E. Nockolds, *J. Biol. Chem.*, **248**, 3313 (1973).
3. D. M. E. Szebenyi, S. K. Obendorf and K. Moffat, *Nature (London)*, **294**, 327 (1981).
4. M. Sundaralingham, R. Bergstrom, G. Strasburg, S. T. Rao, P. Roychowdhury, M. Greaser and B. C. Wang, *Science*, **227**, 945 (1985).
5. O. Herzberg and M. N. G. James, *Nature (London)*, **313**, 653 (1985).
6. Y. S. Babu, J. S. Sack, T. J. Greenhough, C. E. Bugg, A. R. Means and W. J. Cook, *Nature (London)*, **315**, 37 (1985).
7. A. R. Means, J. S. Tash and J. G. Chafouleas, *Physiol. Rev.*, **62**, 1 (1982).
8. C. B. Klee and T. C. Vanaman, *Adv. Protein Chem.*, **35**, 213 (1982).
9. G. N. Phillips, J. P. Fillers and C. Cohen, *J. Mol. Biol.*, **192**, 111 (1986).
10. R. E. Klevit, D. K. Blumenthal, D. E. Wemmer and E. G. Krebs, *Biochemistry*, **24**, 8152 (1985).
11. I. Pilz in *Small-Angle X-ray Scattering*, O. Glatter and O. Kratky, eds. (Academic Press, New York, 1982), pp. 239–293.
12. O. Kratky, *The World of Neglected Dimensions, Small-Angle Scattering of X-rays and Neutrons of Biological Macromolecules* (Deutsche Akademie der Naturforscher Leopoldinn, DDR, 1983).
13. D. B. Heidorn and J. Trewhella, *Biochemistry*, **27**, 909 (1988).
14. B. A. Seaton, J. F. Head, D. M. Engelman and F. M. Richards, *Biochemistry*, **24**, 6740 (1985).
15. J. P. Hennessey, M. Parthasarathy, W. C. Johnson, D. A. Malencik, S. R. Anderson, M. T. Scimerlik and S. Yechiel, *Biopolymers*, **26**, 561 (1987).
16. D. B. Heidorn, S. P. Edmondson, N. W. Downer, R. Kowluru, M. W. Bitenski and J. Trewhella **28**, 2220 (1989).
17. D. C. Laporte, B. M. Weirman and D. Storm, *Biochemistry*, **19**, 3814 (1980).
18. D. R. Marshak, D. M. Watterson and L. J. van Eldick, *Proc. Natl. Acad. Sci. USA*, **78**, 6793 (1981).
19. J. Baudier and D. Gerard, *Biochemistry*, **22**, 3360 (1983).
20. M. Sundaringham, W. Drendel and M. Greaser, *Proc. Natl. Acad. Sci.*, **82**, 944 (1985).
21. C. K. Wang and H. C. Cheung, *J. Mol. Biol.*, **191**, 509 (1986).
22. C.-L. A. Wang, Q. Zhan, T. Tao and J. Gergely, *Biophys. J.*, **49**, 48a (1986).
23. S. F. Mughabghab, *Neutron Cross Sections: Neutron Resonance Parameters and Thermal Cross Sections*, Volume 1, Part B: Z = 61–100 (Academic Press, London, 1984).
24. P. A. Seeger, R. P. Hjelm and M. J. Nutter, these proceedings.