We used site-directed mutagenesis of rabbit muscle aldolase, falling ball viscometry, co-sedimentation binding assays, and negative stain electron microscopy, to identify specific residues involved in the aldolase-actin interaction. Three mutants, R42A (Arg → Ala), K107A (Lys → Ala), and R148A (Arg → Ala), had minimal actin binding activity relative to wild type (wt) aldolase, and one mutant, K229A (Lys → Ala), had intermediate actin binding activity. A mutant with −4,000-fold reduced catalytic activity, D33S (Asp → Ser), had normal actin binding activity. The aldolase substrates and product, fructose 1,6-bisphosphate, fructose 1-phosphate, and dihydroxyacetone phosphate, reversed the gelling of wt aldolase and F-actin, consistent with at least partial overlap of catalytic and actin-binding sites on aldolase. Molecular modeling reveals that the actin-binding residues we have identified are clustered in or around the catalytic pocket of the molecule. These data confirm that the aldolase-actin interaction is due to specific binding, and they suggest that electrostatic interactions between specific residues, rather than net charge, mediate this interaction. Low concentration of wt and D33S aldolase caused formation of high viscosity actin gel networks, while high concentrations of wt and D33S aldolase resulted in solution of the gel by bundling actin filaments, consistent with a potential role for this enzyme in the regulation of cytoplasmic structure.

Most intermediary metabolism is catalyzed by enzymes that are not known to be associated with a discrete organelle or complex, such as the mitochondrion or fatty acid synthase complex. Because of this, metabolic pathways are generally treated as though they exist as a series of diffusion-limited reactions in the aqueous phase of cytoplasm (1). While this assumption simplifies conceptualization and modeling of metabolism, there is substantial evidence that it is oversimplified, and perhaps incorrect, in at least some cases. Hypotheses involving elegant alternative organizational schemes for cellular biochemistry have been proposed over the years (2–12), but have been difficult to test experimentally due to the ephemeral nature of the interactions at this intermediate level of organization (13). These are important hypotheses to test, due to their far-reaching implications for cytoplasmic structure and for metabolic regulation.

Glycolysis is a metabolic pathway that may be organized around the cytoskeleton, rather than in a membrane-bound compartment (14). It has been known for many years that several glycolytic enzymes can interact with cytoskeletal proteins (15) and it has been proposed that some glycolytic enzymes may play structural and/or regulatory roles in cytoplasm, in addition to their catalytic roles (16). Aldolase has one of the highest bound fractions to myofibrils, stress fibers, and F-actin among the glycolytic enzymes (15–19). In fact, aldolase was one of the first actin-binding proteins identified (20, 21). There are multiple binding sites on one aldolase tetramer, demonstrated by its ability to cross-link F-actin into a gel (16, 22, 23). Aldolase binding to F-actin is inhibited by the substrate fructose 1,6-bisphosphate (FBP)1 (16, 23, 24), and its catalytic parameters are also changed when bound to actin or actin containing filaments (24, 25). Both disassembly of the actin cytoskeleton with cytochalasin D and inhibition of glycolytic flux with 2-deoxyglucose result in rapid, reversible release of bound aldolase in 3T3 cells, consistent with physiologically relevant cytoskeletal binding of aldolase in vivo (26).

Several methods have been used in studies aimed at localizing the F-actin binding and catalytic sites on aldolase. Proteolytic cleavage and chemical modification studies indicated spatial separation of substrate and actin-binding sites on aldolase (27, 28). Kinetic studies have shown that actin filaments can modify the catalytic parameters of aldolase (24, 25) and that myofibrils can competitively inhibit FBP cleavage by aldolase (29). More recently, a region of the aldolase molecule bearing sequence similarity to the actin-binding site on actin and actin-binding proteins was identified between residues 33 and 45 of aldolase (30), and it was shown that a synthetic peptide corresponding to aldolase residues 32–52 binds to F-actin and specifically competes with native aldolase for F-actin binding (31).

In this study we have used site-directed mutagenesis, falling ball viscometry (FBV), co-sedimentation binding assays, and negative stain electron microscopy (EM) to identify residues that are involved in the actin binding activity of aldolase. We present evidence that a specific molecular interaction involving several residues in and near the catalytic site of aldolase mediates its actin binding activity, and we show that modifications of single residues on this enzyme can result in significant alterations of its ability to form gel networks with F-actin.
EXPERIMENTAL PROCEDURES

Materials—All biochemicals were purchased from Sigma unless otherwise specified.

Protein Assay—Unless otherwise specified, protein concentrations were measured with Bio-Rad protein assay, using known concentrations of bovine serum albumin as standards.

Site-directed Mutagenesis—Site-directed mutagenesis (32) was performed to change Arg-42 (AGG), Lys-107 (AAG), and Arg-148 (CGT) codons to Ala using the oligodeoxyribonucleotides (50 pmol): 5'-CATCAGAAGGGCGCTGAATCGG-3', 5'-GGTGTCAGCTGGAAGACAGAC-3', and 5'-GCAATTGGGCTTGCGTGCTG, respectively. The Lys-229→Ala mutation, and the Asp-33→Ser mutations, as well as screening, subcloning, and DNA sequence confirmation of all mutations, and protein expression and purification were performed as described previously (33).

Molecular Modeling—The molecular model of aldolase was generated with MOLSCRIPT (34) using coordinates from the 3.0 Å resolution crystal structure of human muscle aldolase (35) on an Indigo workstation (Silicon Graphics, Inc., Mountain View, CA).

Activity Assay—Aldolase activity was determined by measuring the decrease in absorbance/min at 340 nm in a coupled assay (36). Aldolase was diluted in 50 mM Tris-HCl, 1 mM dithiothreitol (DTT), pH 7.5, and added to a cuvette containing 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.16 mM NADH, 10 μg/ml glycerol 3-phosphate dehydrogenase/triosephosphate isomerase. Assays of 1 ml were performed in triplicate at 30°C following addition of substrate over a concentration range of 0.1–10-fold Vmax. Protein concentration was determined by absorbance using 0.01 (1%) = 0.91 mg/ml (37).

Kinetic values were determined from double-reciprocal plots using the least squares method.

Structural Analysis of Recombinant Proteins—Circular dichroism (CD) spectra were determined using a protein concentration of 1.0 mg/ml, which was determined by absorbance at 280 nm, in 1 mM Tris-HCl, 1 mM dithiothreitol (DTT), pH 7.5, at 25°C with an AVIV 60DS spectrometer using a 0.1-cm path length cuvette. Spectra were taken from 180 to 260 nm with the readings averaged for 5 s at each 1-nm increment.

Falling Ball Viscometry—FBV was performed as described previously (38). Briefly, actin was purified from rabbit back and leg muscle by the method of Pardee and Spudich (39) with two polymerization-depolymerization cycles. Ammonium sulfate suspension of rabbit muscle aldolase was dialyzed against 40 mM KCl, 1 mM MgCl2, 0.1 mM DTT, 10 mM imidazole, pH 6.8 (iKMD buffer) overnight. iKMD was used as assay buffer for all experiments. Actin (0.5 or 1 mg/ml), mixed with aldolase or effectors as indicated, was drawn into a 100-μl glass pipette and incubated at 37°C for 30 min in a humidified chamber before viscometry measurements.

Co-sedimentation Assay—100−μl samples were prepared identically as samples for FBV, except that the final incubation was performed in 175-μl polyallomer centrifuge tubes (Beckman Instruments, Inc., Palo Alto, CA) directly. After incubation, the samples were spun in an Airfuge (A-100/18 rotor, Beckman) at 30 ps.i. (−120,000 × g) for 15 min at room temperature. Supernatants and pellets were separated, and pellets were resuspended in iKMD. Samples of supernatant fractions and pellets were run on 10% SDS-polyacrylamide gel electrophoresis and then stained with Comassie Blue before they were scanned with an Arcus II Professional Desktop Scanner (Agfa-Gevaert N.V., Mortsel, Belgium). Quantification was performed with ImageQuant software, version 3.22 (Molecular Dynamics, Sunnyvale, CA).

Negative Stain Electron Microscopy—EM samples were prepared similarly to FBV samples, except that they were incubated as droplets in a Petri dish. After incubation, carbon-coated EM grids were overlaid on the droplets for 1.5 min, then washed with 37°C iKMD, stained with 1% uranyl acetate for 2 min, and dried. Sample grids were examined using a JEM 100-S electron microscope (JEOL USA, Inc., Peabody, MA).

RESULTS

Site-directed Mutants of Aldolase—The five site-directed aldolase mutants we studied varied in both catalytic activity and actin binding activity; the Vmax and Km of wt and mutant aldolases are presented in Table I. The K229A mutant knocked out catalytic activity, D33S decreased Vmax by about 4,000-fold, and the other mutants had smaller effects on Vmax (2–67-fold decreased). The R42A mutant retained catalytic activity closest to that of the wt molecule, with 50% of the wt Vmax and about 3-fold higher Km. The K107A and R148A mutants had the largest increases in Km (9–13-fold).

| Protein | Vmax (units/mg) | Km (μM) |
|---------|----------------|---------|
| wt (Sigma A-1823) | 32.5 ± 0.9 | 7.6 ± 0.7 |
| wt (Bovine Heart) | 20.8 ± 0.5 | 14.3 ± 0.7 |
| D33S (Asp→Ser) | 0.0056 ± 1×10⁻⁶ | 36.5 ± 0.002 |
| R42A (Arg→Ala) | 10.2 ± 0.4 | 45 ± 5 |
| K107A (Lys→Ala) | 0.35 ± 0.01 | 166 ± 12 |
| R148A (Arg→Ala) | 0.31 ± 7×10⁻⁵ | 141 ± 7 |
| K229A (Lys→Ala) | 0* | NA* |

* Assay performed as described by Pagliaro and Taylor (1988).
* Values from Morris and Tolan (1993).
* This value represents <0.002 unit/mg (Morris and Tolan, 1994). NA, not applicable.

Fig. 1 shows the locations of these residues on a MOLSCRIPT model of the 3.0 Å coordinates of human muscle aldolase. Human and rabbit muscle aldolase (aldolase A) share 99% sequence identity, with most of the differing residues in the amino-terminal end of the molecule, and none of them at or near the residues of interest in this report (35, 40, 41). Asp-33, Lys-107, Arg-42, and Lys-229 are located in the central catalytic site, and Arg-42 is just outside the catalytic site pocket. The CD spectra of the wild type and mutant proteins were similar (Fig. 2), indicating that these mutations did not cause major perturbations in the secondary structure. The substantial activity of all three mutant enzymes shown in Fig. 2 further indicated that these mutations did not cause major perturbations in the tertiary structure of the active site.

Arg-42, Lys-107, Arg-148, and Lys-229 Are Important for F-Actin Gel Forming Activity—Wild-type and D33S gelled 0.5 mg/ml F-actin at −0.7 and −0.3 μM, respectively, indicated by a sharp rise in apparent viscosity to >1,500 centipoise (Fig. 3). At high enzyme concentration, however, solation occurred and the viscosity dropped below that of 0.5 mg/ml F-actin alone. Mutants R42A, K107A, and R148A caused minimal viscosity increase up to 3 μM enzyme concentration. K229A was intermediate, causing a slow viscosity increase between 0.5 and 2.5 μM enzyme concentration, after which viscosity started to drop.

We also investigated the effects of FBP, fructose 2,6-bisphosphate, fructose-1-phosphate, fructose-6-phosphate, d-ribose-5-phosphate, glyceroldehyde 3-phosphate, and dihydroxyacetone phosphate on the F-actin gelling activity of wt aldolase. Fig. 4 shows that FBP, DHAP and F1P all inhibited aldolase-F-actin gel formation (1 μM aldolase, 1 mg/ml actin) at less than 5 μM concentrations. Structurally similar compounds F6P, R5P, F-2,6-P, and the aldolase product G3P did not have any detectable effect on aldolase-F-actin gel in the same experimental concentration range, although they eventually did reverse the aldolase-F-actin gel at 500–1,000 μM concentrations (data not shown).

Arg-42, Lys-107, Arg-148, and Lys-229 Are Important for F-Actin Binding Activity—We used a 15-min centrifugation at −120,000 × g to separate free from actin-bound aldolase, and observed different co-sedimentation of wt and mutant aldolases with F-actin (Fig. 5). The co-sedimentation results correlated with FBV data. 3.0 μM wt and D33S aldolase co-pelleted with 1.0 mg/ml F-actin to about the same degree, while less than 10% of R42A, K107A, and R148A co-pelleted with actin; K229A pelleting activity was 30% of wt. Enolase does not gel or bind to F-actin (18, 42) and was used as a negative control (data not shown).

Aldolase Binds F-Actin—The mutant and the substrate inhibition viscometric data are consistent with partial overlap of the aldolase catalytic and actin-binding sites, consistent with previous reports (16, 23, 31). In view of this overlap, we predicted that addition of the substrate FBP to a sol of 10 μM
aldolase and 0.5 mg/ml F-actin would increase viscosity, due to FBP’s ability to compete with actin for aldolase binding and thus lower the effective aldolase concentration so as to permit gelation (Fig. 3). As the FBP concentration continued to increase, more aldolase would be competed off of actin, resulting again in a decrease of viscosity to the baseline. The data in Fig. 6 are consistent with these predictions.

At high aldolase concentrations the F-actin-aldolase gel is solated (Fig. 3), suggesting three possibilities. First, aldolase might sever F-actin (30) and bind to actin monomers or short oligomers, thereby reducing the mass of polymer, and solating the gel (17). Second, aldolase might bundle F-actin, thus reducing the highly cross-linked network of filaments, and induce solation. Third, at high concentration, aldolase might saturate all binding sites on the actin filaments, thereby allowing the gel to solate. The viscosity decrease below that of F-actin alone at high aldolase concentrations (Fig. 3) is inconsistent with the third possibility. We used negative stain EM to distinguish between the first two possibilities. At 1 μM wt aldolase concentration, 0.5 mg/ml F-actin was extensively bundled and cross-linked, as revealed by the formation of dense network of F-actin filaments (Fig. 7A). Actin filaments were also longer and straighter compared to F-actin alone (Fig. 7E). At 3 μM wt aldolase, F-actin was bundled to a higher degree, but these bundles were no longer cross-linked (Fig. 7C). Samples with 1 μM R42A and 0.5 mg/ml F-actin (Fig. 7B) were not distinguishable from those with F-actin alone; when R42A was increased to 3 μM, we observed limited F-actin bundle formation (Fig. 7D).

**Fig. 3. F-actin gelling activity of wt and mutant aldolases.** Different concentrations of wt and mutant aldolases were mixed with 0.5 mg/ml F-actin and incubated at 37 °C for 30 min. before apparent viscosity of the samples were measured with a falling ball viscometer. wt (○), D33S (□), R42A (●), K107A (×), R148A (△), K229A (▲).

**Fig. 4. Effects of phosphosugars on aldolase-F-actin gels.** Different concentrations of reagents were mixed with 2 μM wt aldolase and 1 mg/ml F-actin before viscosity measurements were made. FBP (●), DHAP (○), and F1P (■) solated the gel. F6P (▲), F-2,6-P (□), G3P (△), and R5P (▲) did not solate the gel and are indistinguishable in this figure.

**DISCUSSION**

A Molecular Basis For the Actin Binding Activity of Aldolase—We have used site-directed mutagenesis to identify residues in (Lys-107, Arg-148, and Lys-229) and near (Arg-42) the catalytic site as important sites for the actin binding activity of aldolase. None of these mutations induces significant secondary structural changes in aldolase, as detected with CD spectroscopy (Fig. 2 and Ref. 33). Lys-107, Arg-148, and Arg-42 each appear to be necessary, but not sufficient, for the normal actin binding activity of the wt molecule, since each mutation significantly reduces actin binding and gelling activity. Their location on aldolase confirms that the catalytic site overlaps...
with the actin-binding site topologically as well as functionally (Fig. 1). Previous studies used partial proteolysis to study the actin binding sites on aldolase (27, 31). This approach is very useful in identifying short primary sequences that are important for actin binding, but it cannot identify binding sites on the tertiary conformation of the molecule that result from discontinuous residues in the primary sequence.

The acidic N-terminal region of actin is an attractive candidate for interaction with the positively charged residues we have identified on aldolase. However, there is evidence from a study with affinity-purified polyclonal antibodies that aldolase does not bind to sequence regions 1–7, 18–28, or 40–113 on actin (43). Instead, the same study indicated that aldolase binds to the region around residue 299 on actin (43). In our experiments, aldolase gels F-actin at a stoichiometry of about 1 aldolase/25 actin monomers, so it must occupy a relatively small percentage of the potential aldolase binding sites on actin at this concentration.

Actin Binding and Catalysis: Dual Activities—The ability to virtually knock out catalytic activity with D33S with minimal effect on actin binding activity establishes that these are specific, distinct activities. R42A has no F-actin gelling or binding activity, but high catalytic activity at about half of wt $V_{max}$, while D33S has good F-actin gelling and binding activity but less than 0.04% of wt $V_{max}$, confirming that one of the key sites for actin binding activity (Arg-42) is within residues 32–52 of aldolase, as proposed by O’Reilly and Clarke (31). Arg-42 has been implicated in binding the C6-phosphate of FBP from observations of the crystal structure (44), however, the kinetic evidence of the R42A mutant enzyme does not support this proposal. Residues Arg-42, Lys-107, Arg-148 and Lys-229 are all likely to be positively charged at the experimental pH of 6.8, suggesting that electrostatic binding occurs between aldolase and F-actin. Some substrates and products of aldolase (FBP, F1P, and DHAP) can reverse the binding of wt aldolase to F-actin, consistent with at least partial overlap of catalytic and actin binding sites on aldolase, suggested by the location of Lys-107, Arg-148, and Lys-229 in the catalytic site. Chemical modification studies (40) and site-directed mutagenesis (K107H; Ref. 45) have implicated Lys-107 in C6-phosphate binding. Arg-148 has been implicated in C1-phosphate binding due to its proximity to Lys-146 (46). Characterization of the mutants K107A and R148A are consistent with their roles in C6- and C1-phosphate binding, respectively. Both showed over 10-fold increases in $K_m$ for FBP and a 60 to 70-fold decrease in $V_{max}$. The differences in activity of aldolase toward FBP and F1P have lead to the conclusion that the C1-phosphate binding is stronger than the C6-phosphate binding (47). This is supported by the aldolase-F-actin interactions measured here. F1P reversed aldolase-F-actin gels at more than an order of magnitude lower concentration than F6P; the same was true for the FBP/F-2,6-P pair and the DHAP/G3P pair. These data confirm that FBP, F1P, and DHAP reversal of aldolase-F-actin gelation is not due to nonspecific charge interactions.

The effect of F-actin on the catalytic activity of aldolase has been studied previously. Two early studies showed that F-actin...
significantly to account for the observed increase in the catalytic activity of the other three subunits can be altered is thus unlikely that, by binding to F-actin with one subunit, the conformation or electrostatic configuration of the active site might involve Arg-42, or a residue in that vicinity. The increase in \( V_{\text{max}} \) could be due to effects of binding at this site on the conformation or electrostatic configuration of the active site. Muscle aldolase exists as a tetramer (48), and kinetic evidence indicates that each subunit acts independently (49). It is thus unlikely that, by binding to F-actin with one subunit, the catalytic activity of the other three subunits can be altered significantly to account for the observed increase in \( V_{\text{max}} \). Another possibility is that the active site mutants caused conformational changes in the Arg-42 vicinity which alter their apparent actin binding activity, while in fact the catalytic site is not directly involved in actin binding. This is also unlikely, however, since site-directed mutagenesis of many other residues in or near the active site does not cause major changes in aldolase structure (33, 45, 50).

Implications for Cell Biology—Actin binding activity of enzymes has been implicated in many schemes for non-membrane-bound organization of glycolytic enzymes, but the specificity of these interactions has been open to question. Our results establish that there is a specific site on aldolase responsible for its interaction with actin. This is a significant step in confirming the existence of non-membrane-bound enzyme organization in cytoplasts. The ability of a single residue substitution to dramatically alter the binding characteristics of an enzyme is more consistent with highly organized metabolic machinery than with relatively nonspecific phase separation (51) in cytoplasm. Our data also establish that solution of aldolase-F-actin gels by high concentrations of aldolase is due to bundling activity of aldolase, and that bundling is modulated by the substrate for aldolase, FBP. This behavior is entirely consistent with the concept of functional duality (16), and it provides evidence that a ubiquitous metabolic enzyme may play an integrative role in cytoplasmic organization.

Acknowledgments—We thank Allison Adin, Crystal Batchelor, Ed Reineks, and Fritz Reitz for experimental assistance and helpful discussions. Dr. Harvey Knull for critical comments on the manuscript, Dr. J. Howard for the use of Airugle, Dr. Dan Luchter, John Boykin, and Stephanie Lara for their assistance with electron microscopy, and Dr. Terry Lybrand and the Whittaker Molecular Modeling Laboratory for assistance with molecular modeling.

REFERENCES

1. Stryer, L. (1995) Biochemistry, 4th Ed., pp. 181–206, W. H. Freeman & Co., New York.
2. Wilson, E. B. (1925) The Cell in Development and Heredity, 3rd Ed., Macmillan, New York.
3. Peters, R. A. (1930) Trans. Faraday Soc. 26, 797–809
4. Keleti, T., Batke, J., Ovadi, J., Jancsik, V., and Bartha, F. (1977) Adv. Enzymol. Reg. 15, 233–265
5. Welch, G. R. (1977) Prog. Biophys. Mol. Biol. 32, 103–191
6. Wilson, J. E. (1978) Trends Biochem. Sci. 3, 124–125
7. Clegg, J. S. (1984) Am. J. Physiol. 246, R133–R151
8. Don, M., and Masters, C. J. (1984) Biochim. Biophys. Acta 762, 163–171
9. Pette, D., and Brandau, H. (1964) Biochem. Biophys. Res. Commun. 6, 145–153
10. Srivastava, D. K., and Bernhard, S. A. (1986) Science 234, 1081–1086
11. Pizay, A. G. (1988) FEBS Lett. 237, 1–3
12. Ovadi, J. (1991) J. Theor. Biol. 152, 1–22
13. de Duve, C. (1984) A Guided Tour of the Living Cell, p. 17, Scientific American Books, Inc., New York
14. Clarke, F. M., Stephan, M., Morton, D. J., and Wiedemann, J. (1986) in Regulation of Carbohydrate Metabolism (Beitner, R., ed) Vol. 2, pp. 1–35.
15. CRC Press, Inc., Boca Raton, FL
16. Arnold, H., and Pette, D. (1968) Eur. J. Biochem. 6, 163–171
17. Clarke, F. M., Morton, D. J., Stephan, P., and Wiedemann, J. (1985) Cell Motility: Mechanism and Regulation, pp. 235–250, University of Tokyo Press.
18. Arnold, H., Henning, R., and Pette, D. (1971) Eur. J. Biochem. 22, 121–126
19. Bronstein, W. W., and Knill, H. R. (1981) Can. J. Biochem. 59, 494–499
20. Pagliaro, L. (1995) Adv. Mol. Cell. Biol. 11, 93–123
21. Pette, D., and Brandau, H. (1962) Biochem. Biophys. Res. Commun. 9, 367–370
22. Bauer, A. C., Pette, D., Roisen, F., and Ambriner, W. (1964) Fed. Proc. 23, 310
23. Marsden, J. P., Winzor, D. J., Clarke, F. M., Masters, C. J., and Morton, D. J. (1980) Biochem. J. 186, 89–98
24. Pagliaro, L., and Taylor, D. L. (1988) J. Cell Biol. 107, 981–991
25. Arnold, H., and Pette, D. (1970) Eur. J. Biochem. 15, 350–366
26. Walsh, T. P., Clarke, F. M., and Masters, C. J. (1977) Biochem. J. 165, 165–167
27. Pagliaro, L., and Taylor, D. L. (1992) J. Cell Biol. 118, 859–863
28. Humphreys, L., Reid, S., and Masters, C. (1986) Int. J. Biochem. 18, 7–13
29. Don, M., and Masters, C. J. (1988) Mol. Cell. Biochem. 81, 145–153
30. Harris, S. J., and Winzor, D. J. (1987) Biochim. Biophys. Acta 1191, 121–126
31. Tellam, R. L., Morton, D. J., and Clarke, F. M. (1989) Trends Biochem. Sci. 14, 130–133
32. O'Reilly, G., and Clarke, F. (1993) FEBS Lett. 321, 69–72
33. Taylor, J. W., Ott, J., and Eckstein, F. (1985) Nucleic Acids Res. 13, 8765–8785
34. Morris, A. J., and Tolan, D. R. (1989) J. Biol. Chem. 264, 1095–1100
35. Krajulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
36. Gamblin, S. J., Cooper, B., Millar, J. R., Davies, G. J., Littlechild, J. A., and Watson, H. C. (1990) FEBS Lett. 262, 282–286
37. Ross, E. (1982) J. Biol. Chem. 257, 347–351
38. Baranowsky, T., and Niederland, T. R. (1949) J. Biol. Chem. 180, 543–551
39. Wang, J., Reitz, F., Donaldson, T., and Pagliaro, L. (1994) J. Biochem. Biophys. Methods 268, 251–261
40. Paree, C. D., and Spudich, J. A. (1982) Methods Enzymol. 85, 164–181
41. Lai, C. Y., Nakai, N., and Chang, D. (1974) Science 183, 1204–1206
42. Lai, C. Y. (1975) Arch. Biochem. Biophys. 166, 358–368
43. Pagliaro, L., Ker, K., and Taylor, D. L. (1989) J. Cell Sci. 94, 333–342
44. Méjean, C., Pons, F., Benyamin, Y., and Roustan, C. (1989) Biochem. J. 264, 671–677
45. Gamblin, S. J., Davies, G. J., Grimes, J. M., Jackson, R. M., Littlechild, J. A., and Watson, H. C. (1991) J. Biol. Chem. 266, 573–576
46. Takasaki, Y., Kitajima, Y., Takahashi, I., Sakakibara, M., Muki, T., and Hori, K. (1990) Prog. Clin. Biol. Res. 346, 935–953
47. Hartman, F. C., and Brown, J. P. (1976) Biochem. J. 251, 3057–3062
48. Korenkev, B. L., Tsolas, O., and Lai, C. Y. (1972) Enzymes 7, 213–258
49. Beerman, P. K., and Tolan, D. R. (1994) Protein Sci. 3, 1333–1339
50. Penhoet, E. E., and Rutter, W. J. (1971) J. Biol. Chem. 246, 318–323
51. Morris, A. J., and Tolan, D. R. (1994) Biochemistry 33, 12291–12297
52. Walter, H., and Brooks, D. E. (1995) FEBS Lett. 361, 135–139
The Molecular Nature of the F-actin Binding Activity of Aldolase Revealed with Site-directed Mutants
Jian Wang, Aaron J. Morris, Dean R. Tolan and Len Pagliaro

J. Biol. Chem. 1996, 271:6861-6865.
doi: 10.1074/jbc.271.12.6861

Access the most updated version of this article at http://www.jbc.org/content/271/12/6861

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 47 references, 14 of which can be accessed free at http://www.jbc.org/content/271/12/6861.full.html#ref-list-1