Bean, B. L., Koren, R., & Mildvan, A. S. (1977) *Biochemistry 16*, 3322–3333.
Bloembergen, N., & Morgan, L. (1961) *J. Chem. Phys. 34*, 842.
Duffy, T. H., Saz, H. J., & Nowak, T. (1982) *Biochemistry 21*, 132–139.
Dwek, R. A. (1973) *Nuclear Magnetic Resonance in Biochemistry*, Clarendon Press, Oxford.
Eigen, M., & Hammes, G. (1963) *Adv. Enzymol. Relat. Areas Mol. Biol. 25*, 1–25.
Granot, J., Armstrong, R. N., Kondo, H., Kaiser, E. T., & Mildvan, A. S. (1979) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 38*, 315.
Hebda, C. A., & Nowak, T. (1982a) *J. Biol. Chem. 257*, 5503–5514.
Hebda, C. A., & Nowak, T. (1982b) *J. Biol. Chem. 257*, 5515–5522.
James, T. L. (1975) *Nuclear Magnetic Resonance in Biochemistry*, pp 177–209, Academic Press, New York.
James, T. L., & Cohn, M. (1974) *J. Biol. Chem. 249*, 3519–3526.
Lee, M.-H. (1983) Ph.D. Thesis, University of Notre Dame.
Lee, M.-H., & Nowak, T. (1984) *Biochemistry 23*, 6506–6513.
Li, H., Switzer, R. L., & Mildvan, A. S. (1979) *Arch. Biochem. Biophys. 193*, 1–13.
Makinen, A. L., & Nowak, T. (1983) *J. Biol. Chem. 258*, 11654–11662.
Melamud, E., & Mildvan, A. S. (1975) *J. Biol. Chem. 250*, 8193–8201.
Mildvan, A. S., & Cohn, H. (1970) *Adv. Enzymol. Relat. Areas Mol. Biol. 33*, 1–71.
Mildvan, A. S., & Nowak, T. (1973) *Ann. N.Y. Acad. Sci. 222*, 192–210.
Mildvan, A. S., & Gupta, R. K. (1978) *Methods Enzymol. 49*, 322–358.
Nowak, T. (1978) *Arch. Biochem. Biophy. 186*, 343–350.
Nowak, T. (1981) *Spectrosc. Biochem. 2*, 109–135.
Nowak, T., & Mildvan, A. S. (1972) *Biochemistry 11*, 2819–2828.
Nowak, T., Mildvan, A. S., & Kenyon, G. L. (1973) *Biochemistry 12*, 1690–1701.
Reuben, J., & Cohn, M. (1970) *J. Biol. Chem. 245*, 6539–6546.
Rose, I. A., O'Connell, E. L., Noce, P., Utter, M. F., Wood, H. G., Willard, J. M., Cooper, T. G., & Benziman, M. (1969) *J. Biol. Chem. 244*, 6130–6133.
Solomon, I. (1955) *Phys. Rev. 99*, 559–565.
Solomon, I., & Bloembergen, N. (1956) *J. Chem. Phys. 25*, 261–266.
Villafranca, J. J. (1980) *Dev. Biochem. 10*, 17–30.
Vold, R. L., Waugh, J. S., Klein, M. P., & Phelps, D. E. (1968) *J. Phys. Chem. 48*, 3831–3832.
Watson, D. G., & Kennard, O. (1973) *Acta Crystallogr., Sect. B 29*, 2358–2364.

Opposite Effects of Cofilin and Profilin from Porcine Brain on Rate of Exchange of Actin-Bound Adenosine 5′-Triphosphate†

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ABSTRACT: Cofilin, an actin-binding protein isolated from porcine brain that reacts with actin in a 1:1 molar ratio [Nishida, E., Maekawa, S., & Sakai, H. (1984) *Biochemistry 23*, 5307–5313], decreases the rate of exchange of ATP bound to G-actin with 1,N^6-ethenoadenosine 5′-triphosphate in solution. From analyses of the dependence of the exchange rate on the cofilin concentration under different KCl concentrations, dissociation constants (K_D) for the cofilin–actin binding at 0, 50, and 140 mM KCl were determined to be 0.12, 0.15, and 0.25 μM, respectively. In contrast to cofilin, profilin isolated from porcine brain increases the rate of exchange of G-actin-bound ATP, like *Acanthamoeba* profilin. The kinetic analyses gave K_D values for the profilin–actin binding of 1.1 and 1.5 μM, respectively, at 50 and 200 mM KCl.

Many actin-binding proteins that regulate the state of actin polymerization or higher order structures of actin have been isolated from various tissues and cells (Weeds, 1982; Craing & Pollard, 1982). We have been interested in the regulation of the microfilament system in mammalian tissues and isolated several G-actin-binding proteins from porcine brains by the use of DNase I–agarose affinity chromatography (Nishida et al., 1981; Maekawa et al., 1984).

Cofilin, one of such proteins, has complex effects on actin with the ability to bind to both G- and F-actin (Nishida et al., 1984b). It binds to actin filaments in a 1:1 molar ratio of cofilin to actin monomer in the filament, shortens the average length of the filament, and increases the steady-state concentration of monomeric actins to a limited extent.

It is well-known that G-actin contains 1 mol of bound ATP and that the bound ATP is exchangeable with ATP in solution (Kuehl & Gergely, 1969). Previously, two G-actin-binding proteins have been shown to affect the rate of exchange of actin-bound ATP. DNase I decreases the exchange rate (Mannherz et al., 1980; Hitchcock, 1980), while *Acantha-*

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moeba profilin increases it (Mockrin & Korn, 1980). In addition, from the kinetic analysis the dissociation constant for the binding of G-actin to DNase I or *Acanthamoeba* profilin could be determined. It appears very interesting to see how cofilin affects the exchange of actin-bound ATP. It also seems important to know whether mammalian profilin increases the exchange rate of ATP like *Acanthamoeba* profilin.

In this study, we have investigated the effects of two different actin-binding proteins isolated from porcine brain, cofilin and profilin, on the rate of exchange of actin-bound ATP. These experiments were performed under different KCl concentrations to examine whether the affinity of actin for cofilin or profilin is dependent on the KCl concentration.

Experimental Procedures

Preparation of Proteins. Cofilin, profilin, and brain actin were purified from porcine brain as previously described (Maekawa et al., 1984). Rabbit skeletal muscle actin was prepared by the method of Spudich & Watt (1971) and further purified by gel filtration on Sephadex G-100 equilibrated with a buffer solution containing 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.1 mM DTT,[1] 0.01% NaN_3, and 2 mM HEPES, pH 7.9. Protein concentration was determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard. Actin concentration was determined by UV absorption measurement based on $A^{1\%}_{290} = 6.5$.

Measurement of Exchange of Actin-Bound ATP with ϵATP. 1,N^6-Ethenoadenosine 5′-triphosphate (ϵATP) was purchased from Molecular Probes (Junction City, OR). A stock solution (20 mM ϵATP) was prepared in 2 mM PIPES (pH 7) and diluted in a desired buffer solution before use. G-Actin in the absence or presence of various concentrations of cofilin or profilin was incubated at 20 °C for about 5 min before addition of ϵATP. After the addition (at zero time), the relative fluorescence intensity was followed with time in a temperature-controlled cuvette chamber (at 20 °C) on a Hitachi 650-10S fluorescence spectrophotometer. The excitation and emission wavelengths were 360 and 410 nm, respectively. The fluorescence intensity of ϵATP alone (i.e., in the absence of actin) was subtracted from all the measurement points.

Since it has been shown that the exchange of actin-bound ATP occurs by a unimolecular reaction (Kuehl & Gergely, 1969; Waechter & Engel, 1975) and since all the results obtained in this study showed first-order kinetics irrespective of whether cofilin or profilin was present or not (see Results), the apparent rate constant for the exchange (k_{obsd}) was obtained from a semilogarithmic plot. The logarithm of the difference between the fluorescence intensity at steady state (I_∞) and that at time t (I_t) was plotted against time. In most experiments, the concentration of ϵATP added was ~24 times greater than the concentration of ATP (including actin-bound ATP). Therefore, even if we consider the fact that the affinity constant of ϵATP for actin is about 5 times smaller than that of ATP (Waechter & Engel, 1975), we can say that the apparent rate constant for exchange is nearly equal to the rate constant for dissociation of ATP from actin.

Results

Since the absorption spectrum of ϵATP shifted to a longer wavelength upon its binding to G-actin (Miki et al., 1974), the binding of ϵATP to G-actin can be measured by an increase in the fluorescence intensity at a suitable excitation wavelength. This measurement is sensitive, simple, and fast. Moreover, the technique was successfully applied for the study on the kinetics of exchange of actin-bound ATP (Waechter & Engel, 1975) and for the study dealing with the effect of DNase I on the exchange rate (Mannherz et al., 1980). Therefore, we used this method for investigating the effect of cofilin or profilin on the exchange rate of actin-bound ATP.

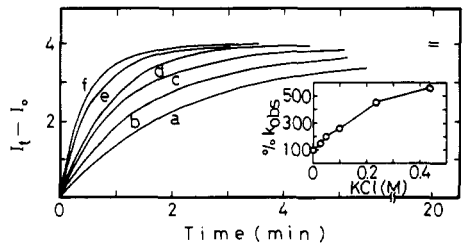

FIGURE 1: Effect of KCl concentration on the rate of exchange of actin-bound ATP with ϵATP. The exchange reaction was initiated by addition of ϵATP (final concentration 60 μM), and the relative fluorescence intensity was measured with time as described under Experimental Procedures. The maximal and minimal levels of the fluorescence intensity among each curve at 20 min are also shown in the figure. Muscle G-actin (0.28 μM) was in a buffer solution of 10 mM PIPES, pH 7.1, 0.1 mM DTT, 3 μM $CaCl_2$, and 2.5 μM ATP containing 0 (a), 25 (b), 50 (c), 100 (d), 240 (e), or 440 mM KCl (f). Each rate constant (k_{obsd}) was obtained from a semilogarithmic plot (not shown) and plotted against the KCl concentration (inset).

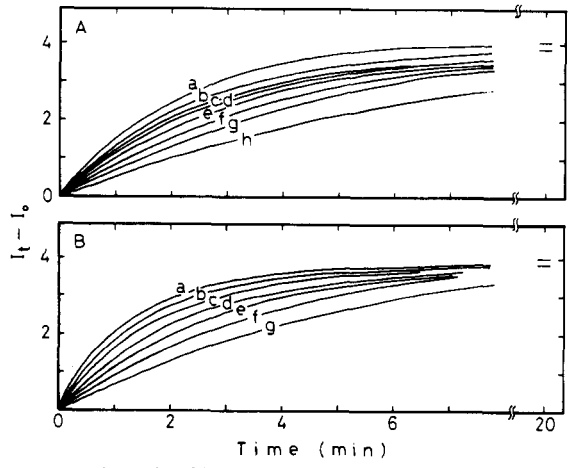

FIGURE 2: Effect of cofilin on the rate of exchange of actin-bound ATP with ϵATP at 0 (A) or 50 mM KCl (B). Measurements were carried out as in Figure 1. The maximal and minimal levels of the fluorescence intensity at 20 min are also shown in the figure. Conditions were the same as in Figure 1 except for KCl concentration. Molar ratios of cofilin to actin: (A) 0 (a), 0.14 (b), 0.20 (c), 0.27 (d), 0.47 (e), 0.67 (f), 1.34 (g), and 2.02 (h); (B) 0 (a), 0.16 (b), 0.32 (c), 0.61 (d), 1.0 (e), 1.4 (f), and 2.0 (g).

Effect of KCl Concentration on Rate of Exchange of Actin-Bound ATP. In order to examine the effect of actin-binding proteins on the exchange of actin-bound ATP in the presence of physiological concentrations of KCl, we need to know the effect of KCl concentration on the ATP exchange. In this experiment, it is necessary to make the actin concentration lower than the critical concentration for polymerization to prevent G-actin from polymerizing. Since it was determined that the critical actin concentration for polymerization was above 0.7 μM in the presence of 50–500 mM KCl (data not shown), the actin concentration used in Figure 1 (0.28 μM) is far below the critical concentration.

Figure 1 clearly shows that the rate of ATP exchange is increased with increasing KCl concentration, but the extent of exchange is not altered. All the curves shown in Figure 1

[1] Abbreviations: DTT, dithiothreitol; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; ϵATP, 1,N^6-ethenoadenosine 5′-triphosphate; PIPES, 1,4-piperazinediethanesulfonic acid.

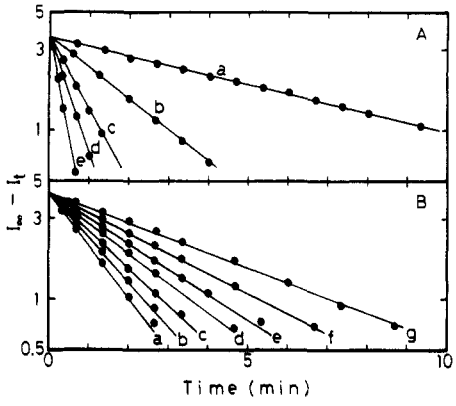

FIGURE 3: Semilogarithmic plots of the data from Figure 6B (A) and from Figure 2B (B). The data were analyzed as described under Experimental Procedures.

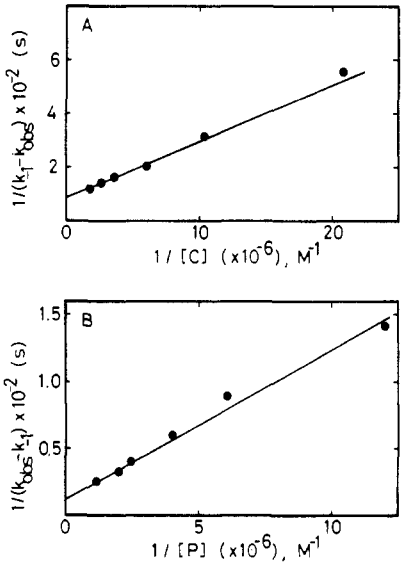

FIGURE 4: Double-reciprocal plots. The data were analyzed as described under Results. (A) Data from Figures 2B and 3B; (B) data from Figure 6A.

followed first-order kinetics (not shown). The rate was about 2.5 times faster in the presence of 0.1 M KCl than in its absence (Figure 1, inset). The result may suggest a KCl-induced conformational change in the G-actin molecule (see Discussion).

Effect of Cofilin on Rate of Exchange of Actin-Bound ATP. Figure 2 shows the effect of increasing concentrations of cofilin on the rate of ATP exchange in 0 (A) and 50 mM KCl (B). It is clear that the rate of exchange is decreased by cofilin in a concentration-dependent manner, but the extent of exchange is not changed. Semilogarithmic plots of the data at various concentrations of cofilin fitted well with the straight lines in both 0 (not shown) and 50 mM KCl (Figure 3B). The apparent rate constant (k_{obsd}) was obtained from the slope of each line.

As reported by Mockrin & Korn (1980) in their study on the effect of *Acanthamoeba* profilin on the rate of ATP exchange, the value for the rate constant for the dissociation of ATP from the cofilin–actin–ATP complex (k_{-2}) can be determined by a double-reciprocal plot [$1/(k_{-1} - k_{obsd})$ vs. $1/[C]$, where C is cofilin and k_{-1} is the rate constant for the ATP dissociation from the actin–ATP complex], whose y axis intercept is $1/(k_{-1} - k_{-2})$. In the case of the experiment carried out in 50 mM KCl, the value for k_{-1}, which was obtained from line a of Figure 3B, was 1.2×10^{-2} s^{-1}. The double-reciprocal plot [$1/(k_{-1} - k_{obsd})$ vs. $1/[C]$] fitted well with a straight line

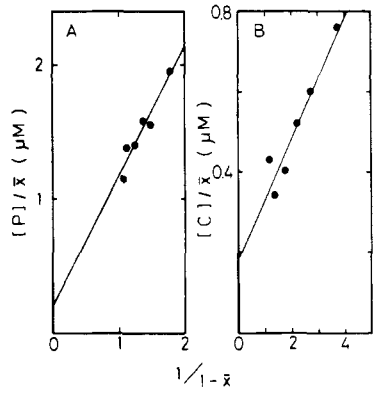

FIGURE 5: Modified Scatchard plots. The data were analyzed as described under Results. (A) Data from Figures 6A and 4B; (B) data from Figures 2B, 3B, and 4A.

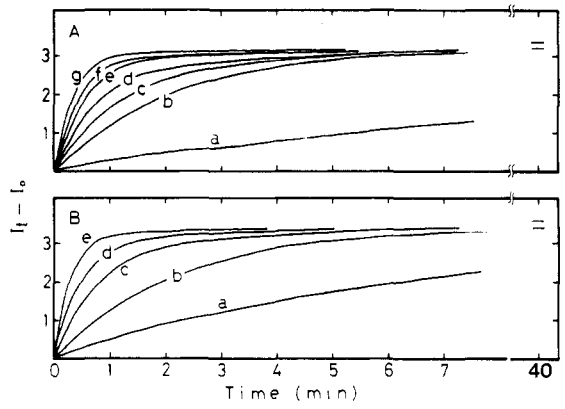

FIGURE 6: Effect of profilin on the rate of exchange of actin-bound ATP with εATP at 50 (A) or 200 mM KCl (B). Measurements were carried out as in Figure 1. The maximal and minimal levels of the fluorescence intensity at 40 min are also shown in the figure. Muscle G-actin (0.27 μM) was in a buffer solution of 9 mM PIPES, 0.5 mM HEPES, pH 7.1, 0.1 mM DTT, and 2.5 μM ATP containing 50 mM KCl and 0.17 mM $CaCl_2$ (A) or 200 mM KCl and 0.14 mM $CaCl_2$ (B). Since preliminary experiments demonstrated that profilin increased the exchange rate, the $CaCl_2$ concentration was increased to 0.14–0.17 mM from 3 μM in Figures 1 and 2 to slow down the exchange rate (Kuehl & Gergely, 1969). Molar ratios of profilin to actin: (A) 0 (a), 0.33 (b), 0.63 (c), 0.93 (d), 1.6 (e), 1.9 (f), and 3.1 (g); (B) 0 (a), 0.18 (b), 0.46 (c), 0.93 (d), and 1.8 (e).

(Figure 4A). The value for k_{-2}, the difference between the value for k_{-1} and the reciprocal of the y-axis intercept of this line, was calculated to be 0.9×10^{-3} s^{-1}. From these values, the fractional activity [$\bar{x} = (k_{-1} - k_{obsd})/(k_{-1} - k_{-2})$] was determined for each cofilin concentration. Then, the data were analyzed by a modified Scatchard plot:

$$[C_T]/\bar{x} = n[A_T] + K_D[1/(1 - \bar{x})]$$

where $[C_T]$ = total cofilin concentration, $[A_T]$ = total actin concentration, K_D = dissociation constant, and n = moles of cofilin bound per mole of actin. A plot of $[C_T]/\bar{x}$ vs. $1/(1 - \bar{x})$ is shown in Figure 5B. From this line, $K_D = 0.15$ μM (the slope) and $n = 0.68$ (y-axis intercept/$[A_T]$). In another series of experiment under the same conditions, the same analysis gave $K_D = 0.18$ μM and $n = 0.87$.

K_D and n for the cofilin–actin binding at 0 mM KCl were determined to be 0.12 μM and 0.64, respectively, by the same analytical procedure as above. At 140 mM KCl, $K_D = 0.25$ μM and $n = 0.82$. Thus, the binding of cofilin to monomeric actin is sensitive to the KCl concentration.

Effect of Profilin on Rate of Exchange of Actin-Bound ATP. Figure 6 shows the effect of increasing concentrations of profilin on the rate of ATP exchange in 50 (A) and 200

mM KCl (B). Profilin increased the rate of exchange in a concentration-dependent manner but did not change the maximal exchange. Semilogarithmic plots of the data fitted well with the straight lines (Figure 3A).

The same analytical procedure as above was applied for these data. One example of a double-reciprocal plot [$1/(k_{obsd} - k_{-1})$ vs. $1/[P]$, where P is profilin] is shown in Figure 4B, and a modified Scatchard plot is shown in Figure 5A. From this, at 50 mM KCl, K_D for the profilin–actin binding and moles of profilin bound per mole of actin (n) were determined to be 1.1 μM and 0.70, respectively. At 200 mM KCl, K_D = 1.5 μM and n = 0.91.

In one experiment, the effect of profilin on the exchange rate of ATP bound to brain actin instead of muscle actin was examined at 25 mM KCl (not shown). It was found that profilin also increases the rate of exchange of brain actin bound ATP. By analyzing the data by a modified Scatchard plot, the value for K_D was determined to be about 0.5 μM.

Discussion

Cofilin binds to both G- and F-actins and modulates both the polymerization state and the structure of actin filaments (Nishida et al., 1984b). Although the previous paper documented that cofilin binds to actin filaments in a 1:1 molar ratio of cofilin to actin monomer in the filament and that cofilin and G-actin form a 1:1 complex at low ionic strength, little was known about the interaction of cofilin with monomeric actin in the presence of physiological concentrations of KCl.

In this study, we have shown that cofilin decreases the rate of exchange of actin-bound ATP in the absence and presence of various concentrations of KCl, with a low concentration of monomeric actin (0.28 μM) that is far below the critical actin concentration for polymerization. Moreover, the values for K_D at 0, 50, and 140 mM KCl were determined to be 0.12, 0.15, and 0.25 μM, respectively, from the analysis of the dependence of the exchange rate of ATP on the cofilin concentration. These results clearly demonstrate that at physiological ionic strength cofilin is capable of binding to monomeric actin with a relatively high affinity constant. The results further indicate that the cofilin–actin binding is dependent on the KCl concentration. This is consistent with the experimental result that cofilin can be eluted from the DNase I–agarose column with a high ionic strength buffer solution (Maekawa et al., 1984).

In contrast to cofilin, brain profilin was, here, shown to increase the rate of exchange of actin-bound ATP in the presence of 25–200 mM KCl. The result suggests that purified profilin is capable of binding to monomeric actin under physiological salt conditions. From the kinetic analyses, the values for K_D were determined to be 1.1–1.5 μM for muscle actin and $\sim$0.5 μM for brain actin.

Previously, Mockrin & Korn (1980) demonstrated that *Acanthamoeba* profilin increases the rate of ATP exchange of muscle actin at low ionic strength. The K_D for the binding of *Acanthamoeba* profilin to muscle actin was, then, determined to be 30–50 μM. This value is much higher than the K_D (1.1–1.5 μM) for the brain profilin–muscle actin binding just described above. Recently, Tobacman & Korn (1982) and Tseng & Pollard (1982) showed from the studies on the effect of *Acanthamoeba* profilin on actin polymerization that the K_D value for the interaction of *Acanthamoeba* profilin with *Acanthamoeba* actin is about 2–8 μM. Although this value is much lower than the K_D (50 μM) for the *Acanthamoeba* profilin–muscle actin interaction, it is still higher than the K_D values for the interaction of brain profilin with either muscle actin (1.1–1.5 μM) or brain actin (0.5 μM) determined in this study. Therefore, it is probable that mammalian brain profilin has a higher affinity for actins than *Acanthamoeba* profilin. This correlates well with the fact that brain profilin inhibits actin polymerization more strongly than does *Acanthamoeba* profilin (Nishida et al., 1984a; Tobacman & Korn, 1982; Tseng & Pollard, 1982). In spite of these quantitative differences, *Acanthamoeba* and brain profilins exhibit qualitatively the same effects both on actin polymerization and on the exchange of actin-bound ATP. Therefore, both proteins are reasonably called profilin.

It has been proposed that a conformational change in the G-actin molecule occurs on addition of 0.1 M KCl. This has been supported by several experiments such as the proteolysis experiments (Rich & Estes, 1976), UV absorption difference spectroscopy (Rouayrenc & Travers, 1981; Pardee & Spudich, 1982), the studies with fluorescently labeled actin (Frieden et al., 1980), and the changes in the affinity of actin for ATP (Pardee & Spudich, 1982). Although the former two experiments have been questioned recently by Fisher et al. (1983), the latter two still support the above proposal. The present result that the rate of exchange of actin-bound ATP was increased with increasing KCl concentration appears to provide additional evidence for the KCl-induced change in actin molecule. Neidl & Engel (1979) also observed a gradual increase in the rate of ATP exchange on addition of NaCl up to 64 mM. Therefore, a subtle and local change, not a gross conformational change (Barden et al., 1983), in actin molecule may occur on addition of KCl or NaCl.

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Registry No. ATP, 56-65-5.

References

Barden, J. A., Wu, C.-S. C., & dos Remedios, C. G. (1983) *Biochim. Biophys. Acta 748*, 230–235.

Craig, S. W., & Pollard, T. D. (1982) *Trends Biochem. Sci. (Pers. Ed.) 7*, 88–92.

Fisher, A. J., Curmi, P. M. G., Barden, J. A., & dos Remedios, C. G. (1983) *Biochim. Biophys. Acta 748*, 220–229.

Frieden, C., Lieberman, D., & Gilbert, H. R. (1980) *J. Biol. Chem. 255*, 8991–8993.

Hitchcock, S. E. (1980) *J. Biol. Chem. 255*, 5668–5673.

Kuehl, W. M., & Gergely, J. (1969) *J. Biol. Chem. 244*, 4720–4729.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Maekawa, S., Nishida, E., Ohta, Y., & Sakai, H. (1984) *J. Biochem. (Tokyo) 95*, 377–385.

Mannherz, H. G., Goody, R. S., Konrad, M., & Nowak, E. (1980) *Eur. J. Biochem. 104*, 367–379.

Miki, M., Ohnuma, H., & Mihashi, K. (1974) *FEBS Lett. 46*, 17–19.

Mockrin, S. C., & Korn, E. D. (1980) *Biochemistry 19*, 5359–5362.

Neidl, C., & Engel, J. (1979) *Eur. J. Biochem. 101*, 163–169.

Nishida, E., Kuwaki, T., Maekawa, S., & Sakai, H. (1981) *J. Biochem. (Tokyo) 89*, 1655–1658.

Nishida, E., Maekawa, S., & Sakai, H. (1984a) *J. Biochem. (Tokyo) 95*, 399–404.

Nishida, E., Maekawa, S., & Sakai, H. (1984b) *Biochemistry 23*, 5307–5313.

Pardee, J. D., & Spudich, J. A. (1982) *J. Cell Biol. 93*, 648–654.
Rich, S. A., & Estes, J. E. (1976) *J. Mol. Biol. 104*, 777–792.
Rouayrenc, J.-F., & Travers, F. (1981) *Eur. J. Biochem. 116*, 73–77.
Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Tobacman, L. S., & Korn, E. D. (1982) *J. Biol. Chem. 257*, 4166–4170.
Tseng, P. C.-H., & Pollard, T. D. (1982) *J. Cell Biol. 94*, 213–218.
Waechter, F., & Engel, J. (1975) *Eur. J. Biochem. 57*, 453–459.
Weeds, A. G. (1982) *Nature (London) 296*, 811–816.

Amino Acid Sequence of the Amphiphilic Phosphocarrier Protein Factor III^{Lac} of the Lactose-Specific Phosphotransferase System of *Staphylococcus aureus*†

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ABSTRACT: The lactose-specific factor III of the phosphotransferase system of *Staphylococcus aureus* is an amphiphilic trimeric protein composed of identical subunits. It is hydrophilic in its unphosphorylated state and can be isolated from the cytoplasmic protein fraction. It becomes a constituent of the membrane-bound phosphotransferase complex upon phosphorylation of a single histidyl residue. The sequence of *S. aureus* factor III^{Lac} was determined and revealed that the subunits consist of 103 residues corresponding to a M_r of 11 367 and of 34 101 for the native trimer: Met-Asn-Arg-Glu-Glu-Val-Gln-Leu-Gly-Phe-Glu-Ile-Val-Ala-Phe-Ala-Gly-Asp-Ala-Arg-Ser-Lys-Phe-Leu-Glu-Ala-Leu-Thr-Ala-Ala-Gln-Ala-Gly-Asp-Phe-Ala-Lys-Ala-Asp-Ala-Leu-Ile-Glu-Glu-Gly-Asn-Asn-Cys-Ile-Ala-Gln-Ala-His-Arg-Ala-Gln-Thr-Ser-Leu-Leu-Ala-Lys-Glu-Ala-Gln-Gly-Asp-Asp-Ile-Ala-Tyr-Ser-Val-Thr-Met-Met-His-Gly-Gln-Asp-His*-Leu-Met-Thr-Thr-Ile-Leu-Leu-Lys-Asp-Leu-His-Lys-Lys-Leu-Leu-Glu-Phe-Tyr-Lys-Arg-Gly. According to this sequence and previous work histidine residue 82 located in the C-terminal part of the polypeptide chain is phosphorylated at the N-3 position by phosphoenolpyruvate, enzyme I, and histidine-containing phosphocarrier protein. The N-terminal part of the protein comprising approximately one-third of the chain exhibits in vitro affinity toward membrane-bound enzyme II^{Lac}.

The phosphotransferase system (PTS)[1] is the major active transport system for carbohydrates and is extensively studied at the biochemical level (Roseman et al., 1982). Recent genetic and biochemical studies show that this multienzyme system is also involved in regulation (Dills et al., 1980).

The lactose-specific PTS of *Staphylococcus aureus* consists of the inducible proteins factor III^{Lac} and enzyme II^{Lac} and the constitutively expressed proteins enzyme I and HPr. These four proteins interact as shown by the following reaction scheme:

$$\text{PEP} + \text{enzyme I} \xrightleftharpoons{Mg^{2+}} \text{P–enzyme I} + \text{pyruvate} \quad (1)$$

$$\text{P–enzyme I} + \text{HPr} \rightleftharpoons \text{P–HPr} + \text{enzyme I} \quad (2)$$

$$3\text{P–HPr} + \text{factor III}^{Lac} \rightleftharpoons \text{P}_3\text{–factor III}^{Lac} + \text{HPr} \quad (3)$$

$$\text{P}_3\text{–factor III}^{Lac} + 3\text{lactose} \xrightarrow[\text{enzyme II}^{Lac}]{Mg^{2+}} 3\text{lactose-6-P} + \text{factor III}^{Lac} \quad (4)$$

As already reported earlier, the structure of the factor III^{Lac} protein is of great interest since it catalyzes the phosphotransfer between the water phase of the cytoplasm (eq 1–3) and the lipid phase of the membrane (eq 4). We therefore designated the protein a phase-transfer catalyst (Deutscher et al., 1982). One of the unusual properties of the protein that supports this feature is to occur in the phosphorylated form in a conformation with increased hydrophobicity. The hydrophobic properties of this protein are conferred by the first 40 residues of the amino-terminal sequence. This region also carries the binding site for the factor III–enzyme II interaction. The active center peptide isolated earlier in the phosphorylated and nonphosphorylated forms is located on a separate functional domain. The elucidation of the primary structure of factor

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[1] Abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; DABITC, 4-(*N*,*N*-dimethylamino)-4′-isothiocyanatoazobenzene; PTS, phosphotransferase system; FIII^{Lac}, factor III specific for lactose; P-FIII^{Lac}, FIII^{Lac} phosphorylated at His-82; HPr, histidine-containing phosphocarrier protein; P-HPr, HPr phosphorylated at His-15; PTH, phenylthiohydantoin; HPLC, high-pressure/performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid; PEP, phosphoenolpyruvate; Tris, tris(hydroxymethyl)-aminomethane.

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