Conformation-Dependent Acetylation and Nitration of the Protein Activator of Cyclic Adenosine 3′,5′-Monophosphate Phosphodiesterase. Selective Nitration of Tyrosine Residue 138†

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ABSTRACT: The conformational transition accompanying the binding of Ca^{2+} to the Ca^{2+}-dependent activator protein of cyclic adenosine 3′,5′-monophosphate phosphodiesterase is reflected in the reactivity of the tyrosine residues with *N*-acetylimidazole. In the presence of Ca^{2+} (8×10^{-4} M), only tyrosine residue 99 is acetylated while in the presence of [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA) (10^{-3} M) both tyrosine residues (99 and 138) are acetylated. After reaction with *N*-acetylimidazole (in the presence of Ca^{2+} or EGTA), activator protein showed a 30% loss in ϵ-amino groups as determined by quantitative ninhydrin analysis. Acetylation of activator protein results in a decrease (two- to fourfold) in its affinity for phosphodiesterase that is not restored after deacetylation of the tyrosine residues. The greatest loss in affinity (fourfold) was obtained in the preparation of activator protein acetylated in the presence of Ca^{2+}. These results indicate that acetylation of the lysine residues of activator protein is responsible for the loss in affinity for enzyme. Reaction of activator protein, in the presence of Ca^{2+}, first with *N*-acetylimidazole (at pH 7.5) and then with tetranitromethane (at pH 8.0) leads to selective nitration of tyrosine residue 138. Spectrophotometric titration of nitrotyrosine residue 138 in the presence of Ca^{2+} gave an apparent p*K* of 8.6 which is shifted to 8.1 in the presence of EGTA. The apparent p*K* of 7.3 for nitrotyrosine residue 99 is not changed by removal of Ca^{2+}. These results taken with others [Richman, P. G., & Klee, C. B. (1978) *Biochemistry 17*, 928] indicate that tyrosine residue 99 is exposed to solvent and its microenvironment is not substantially altered by Ca^{2+} binding to the protein. Tyrosine residue 138, in contrast, is in a unique microenvironment that is substantially altered by Ca^{2+} binding.

Although activator protein[1] was first detected, purified, and characterized as an activator of cAMP[2] phosphodiesterase (see Wang et al., 1975, for a recent review), it has recently been shown to be a multifunctional protein. Thus, activator protein can replace troponin C in the activation of actomyosin ATPase (Amphlett et al., 1976; Dedman et al., 1977a), it activates adenylate cyclase (Brostrom et al., 1975; Cheung et al., 1975) and more recently it was shown to activate a Ca^{2+}-dependent ATPase from erythrocyte membranes (Jarrett & Penniston, 1977; Gopinath & Vincenzi, 1977). The experimental evidence indicates that binding of Ca^{2+} to activator protein is the initial event in these interactions as well as in its interaction with cAMP phosphodiesterase (Kakiuchi et al., 1973; Teo & Wang, 1973; Lin et al., 1974, 1975; Wolff et al., 1977).

Upon binding of Ca^{2+}, activator protein undergoes alterations in its secondary and tertiary structure as seen by changes in its fluorescence, circular dichroism, and UV absorption spectra, increased resistance to trypsin, and changes in its reactivity toward group specific chemical modification reactions (Wang et al., 1975; Liu & Cheung, 1976; Klee, 1977; Wolff et al., 1977; Walsh & Stevens, 1977; Dedman et al., 1977b; Richman & Klee, 1978).

Activator protein contains two tyrosine residues and they are at positions 99 and 138 of the polypeptide chain (Vanaman et al., 1977). Results previously reported from this laboratory indicated that tyrosine-99 is selectively nitrated by tetranitromethane in the presence of EGTA (Richman & Klee, 1978). In the presence of Ca^{2+} both tyrosine residues are nitrated. This finding and several other considerations led us to postulate that tyrosine-138 is in a unique microenvironment that is substantially altered upon binding of Ca^{2+} to activator protein.

This communication deals with the specific chemical modification of activator protein with the production of well-characterized spectral probes (e.g., nitrotyrosine residues) that can be utilized in studying the interaction of specific residues of activator protein with Ca^{2+}, and with the enzymatic systems under its control. Conformation-dependent reaction of activator protein with *N*-acetylimidazole has further elucidated the nature of the separate microenvironments of the two tyrosine residues. In addition, by combination of acetylation with *N*-acetylimidazole and nitration with tetranitromethane under defined conditions, tyrosine residue 138 was selectively nitrated.

Materials and Methods

N-Acetylimidazole was a product of Pierce (mp 98–100 °C, uncorrected). Activator protein was purified from bovine brain acetone powder (Pel-freeze) as previously described (Klee, 1977). Activator-dependent cAMP phosphodiesterase was obtained as previously described (Richman & Klee, 1978). All other reagents were the purest grades commercially available.

Reactions of activator protein (2.3 mg/mL) with *N*-acetylimidazole (60-fold excess over tyrosine) were performed in 0.05 M Hepes buffer (pH 7.5) at 23 °C in final reaction volumes of 0.5 mL. Other additions are indicated below. Reactions were initiated by addition of a freshly prepared solution of *N*-acetylimidazole. After 90 min the reaction mixtures were

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[1] Activator protein is also called modulator protein and Ca^{2+}-dependent regulatory protein.

[2] The abbreviations used are: UV, ultraviolet; cAMP, cyclic adenosine monophosphate; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; mol wt, molecular weight.

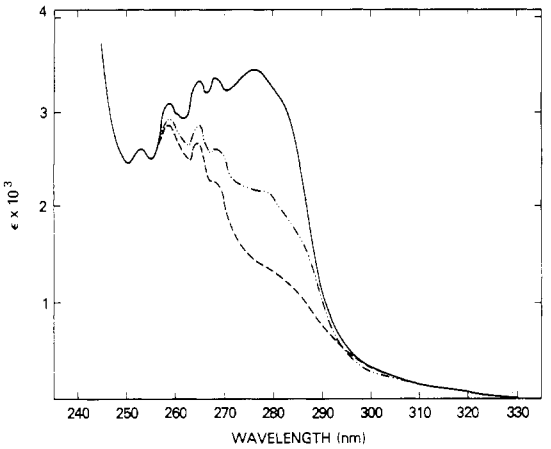

FIGURE 1: UV spectra of activator protein after reaction with *N*-acetylimidazole. Activator protein was reacted at pH 7.5 with *N*-acetylimidazole (60-fold excess over tyrosine) and then dialyzed against 0.02 M Hepes buffer (pH 6.7) (see text for details). Prior to running spectra, samples were centrifuged at 17 000*g* (4 °C) for 45 min to remove any extraneous material. Protein concentrations were calculated from the A_{253nm}, after correction for light scattering (Wetlaufer, 1962), utilizing an ϵ_{253nm} = 2050 (Klee, 1977). The three spectra represent activator protein reacted in the presence of Ca^{2+} (— · · —), EGTA (- - -), or mock-reacted "control" (—).

dialyzed (8000 mol wt cut-off tubing, Thomas) against 0.02 M Hepes buffer (pH 6.7) at 4 °C.

Measurements of absorbance, spectrophotometric titrations and reactions of activator protein with tetranitromethane were performed as previously described (Richman & Klee, 1978). cAMP phosphodiesterase assays were performed as previously described (Klee, 1977), except that 0.04 M Hepes buffer (pH 7.5) was used instead of Tris buffer (pH 8). The concentration of cAMP in the assay reaction mixtures was 2×10^{-4} M.

Results

Reaction of Activator Protein with N-Acetylimidazole. Activator protein was reacted with *N*-acetylimidazole as described under Materials and Methods, in the presence of either EGTA (1×10^{-3} M) or Ca^{2+} (8×10^{-4} M). In addition, a control sample of activator protein was subjected to the same reaction conditions with the omission of *N*-acetylimidazole. The UV spectra of the three reaction mixtures after dialysis are shown in Figure 1. The control curve shows the spectrum of unmodified activator protein. The other spectra exhibit the decreased absorption in the 270–285 nm region characteristic of acetylation of tyrosine hydroxyl groups (Riordan & Vallee, 1972a). The extent of O-acetylation calculated from these data is given in Table I and is much greater in the presence of EGTA than in the presence of Ca^{2+}.

The ability of the two preparations of *O*-acetyltyrosyl activator protein to activate activator-dependent cAMP phosphodiesterase was examined. Both preparations of acetylated activator protein (Table I) gave the same maximal activation of cAMP phosphodiesterase as that obtained with "control" activator[3] (data not shown). However, the apparent K_m values (concentration of activator protein that gave half-maximal activation) were increased over that of "control" activator (Table I). Paradoxically, the preparation with only one tyrosine residue acetylated showed the greatest increase in apparent K_m.

In addition to acetylation of tyrosine residues, *N*-acetylimidazole has been shown to acetylate ϵ-amino and sulfhydryl groups of proteins (Riordan et al., 1965). Since activator protein does not contain any cysteine residues the analyses were confined to the ϵ-amino group. Quantitative ninhydrin analyses (Moore & Stein, 1954) were performed on the two preparations of acetylated activator protein. Both preparations of O-acetylated activator protein exhibited a 30% decrease in ϵ-amino groups from the "control" preparation.

[3] "Control" activator behaved in all respects like unmodified activator protein.

TABLE I: Extent of Acetylation of Activator Protein and Its Effect on Apparent K_m for cAMP Phosphodiesterase.

additions to reaction	*O*-acetyltyrosine[a] (residues/mol)	apparent $K_m \times 10^9$ [b] (M)	
		O-acetylated	deacetylated[c]
mock-reacted "control"	0	2.5 ± 0.3 (2)	2.2 ± 0.3 (2)
Ca^{2+}	1.07	10.3 ± 2.3 (2)	8.3 ± 2.7 (2)
EGTA	1.77	4.7 ± 0.3 (2)	4.9 ± 0.1 (2)

[a] Values calculated from the data in Figure 1, utilizing a $\Delta\epsilon_{278nm}$ of −1160 (Riordan & Vallee, 1965) for O-acetylation of tyrosine. [b] The enzyme was assayed as described under Materials and Methods in the presence of 10^{-9} to 10^{-6} M of the indicated preparations of acetylated activator proteins. The apparent K_m was taken as the concentration of activator protein that gave half-maximal activation. The number of determinations is indicated by the numbers in parentheses. [c] The tyrosyl residues were deacetylated by reaction (23 °C) with 0.1 M NH_2OH at pH 8 (Riordan & Vallee, 1972b). When there was no further increase in A_{278nm} (about 10 min), the protein was subjected to gel filtration to remove the NH_2OH. After this treatment the preparation of activator acetylated in the presence of Ca^{2+} (and the "control") showed the UV spectra of unreacted activator protein. The preparation acetylated in the presence of EGTA gave a $\Delta\epsilon_{278nm}$ = 1160 and 1792 when deacetylated in the presence of Ca^{2+} or EGTA, respectively. The protein deacetylated in the presence of EGTA was utilized for activity measurements.

Mild deacetylation of the tyrosine residues failed to restore the apparent K_m values of activator protein for enzyme to normal ("control") levels (Table I). The results indicate that the decrease in affinity of activator protein for phosphodiesterase seen after reaction of activator protein with *N*-acetylimidazole is a consequence of acetylation of the ϵ-amino groups rather than the tyrosine groups.

Selective Nitration of Tyrosine Residue 138 of Activator Protein. The *O*-acetyltyrosyl activator protein prepared in the presence of Ca^{2+} has 1 mol of its tyrosine residues acetylated per mol of protein (Table I). This could reflect partial acetylation of each of the two tyrosine residues or, alternatively, acetylation of a specific tyrosine residue. Since O-acetylated tyrosine residues are resistant to nitration with tetranitromethane (Riordan et al., 1967), the nitration of the residual tyrosine residue(s) can be used as a tool to distinguish between the two alternatives [tyrosine residue 138 of activator protein can be nitrated only in the presence of Ca^{2+} (Richman & Klee, 1978)].

O-Acetyltyrosyl activator protein prepared in the presence of Ca^{2+} was chromatographed on a column of Sephadex G-25 equilibrated with 0.01 M Tris buffer (pH 8.0). The protein eluted from the column was immediately reacted (23 °C) with tetranitromethane for 1 h in the presence of either 10^{-3} M EGTA or 10^{-3} M Ca^{2+} as previously described (Richman & Klee, 1978). After Sephadex chromatography of the reaction mixtures the extent of nitration was determined spectrally utilizing an ϵ_{381nm} of 2200 (Riordan & Vallee, 1972b). Protein concentration was determined as described by Lowry et al. (1951) (mol wt 16 500) using bovine serum albumin as the

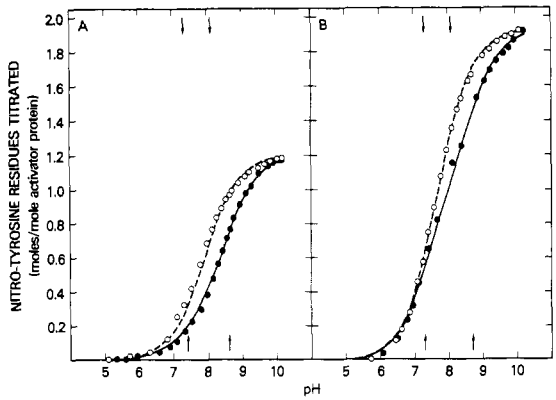

FIGURE 2: Spectrophotometric titration of nitrotyrosyl activator protein. Activator protein preparation preferentially nitrated at position 138 after prior acetylation (in the presence of Ca^{2+}) (panel A) or nitrated (without prior acetylation) at positions 99 and 138 (panel B) were titrated in 0.1 M KCl, as previously described (Richman & Klee, 1978), in the presence of either 1.0×10^{-4} M EGTA (○ — ○) or 1.0×10^{-3} M Ca^{2+} (● — ●). Protein concentrations were 0.5 mg/mL. The curves were calculated (Henderson-Hasselbalch equation) assuming 0.32 mol of nitrotyrosine residue 99 and 0.87 mol of nitrotyrosine residue 138 (panel A) based on the data given in the text, and 1.00 mol of nitrotyrosine residue 99 and 0.92 mol of nitrotyrosine residue 138 (panel B) as previously reported (Richman & Klee, 1978). The arrows indicate the pK values used in calculating the respective curves.

standard. There were 1.19 and 0.32 nitrotyrosine residues produced per mol in the presence of Ca^{2+} or EGTA, respectively.

Under the same conditions as above (Richman & Klee, 1978), unmodified activator protein gives 1.92 and 1.11 nitrotyrosine residues per mol in the presence of Ca^{2+} or EGTA, respectively. These results suggest that tyrosine-138 was the nonacetylated residue (see also titration results below).

The fact that there was greater than 1 mol of nitrotyrosine per mol of activator produced in the presence of Ca^{2+} suggests that some deacetylation occurred during the reaction of *O*-acetyltyrosyl activator protein with tetranitromethane. There was no change in the A_{278nm} of this preparation of *O*-acetyltyrosyl activator protein in a control reaction mixture[4] after 1 h at 23 °C. The mechanism of the observed deacetylation is not clear from the available data. The results suggest that in the presence of Ca^{2+} tyrosine residue 99 was selectively acetylated with *N*-acetylimidazole, allowing subsequent preferential nitration of tyrosine residue 138.

Effect of Ca^{2+} Binding on the Apparent pK of Nitrotyrosine Residue 138 of Activator Protein. It was previously demonstrated that the 3-nitro derivative of tyrosine-138 of activator protein titrates with an apparent pK of 8.6 in the presence of Ca^{2+} and is thereby distinguishable from nitrotyrosine-99 (apparent pK = 7.3 in the presence or absence of Ca^{2+}) (Richman & Klee, 1978). When the nitrotyrosyl activator prepared after acetylation (see above) was spectrophotometrically titrated in the presence of Ca^{2+}, an apparent pK of 8.6 was obtained (Figure 2A). The apparent pK in the presence of Ca^{2+} agrees well with the value previously assigned to nitrotyrosine-138. However, in the presence of EGTA the apparent pK was shifted to 8.1. The titration curve in the presence of EGTA or Ca^{2+} for activator protein containing 1.92 mol of nitrotyrosine per mol (Richman & Klee, 1978) is shown in Figure 2B. In agreement with the results in Figure 2A, the apparent pK of nitrotyrosine-138 is shifted to the left in the presence of EGTA. There is no effect of Ca^{2+} binding on the apparent pK of nitrotyrosine-99 (Figure 2B) in agreement with results previously reported. Note that prior acetylation of activator protein does not affect the apparent pKs of the nitrotyrosine residues. The titration results also confirm the assignment of tyrosine residue 138 as the principal site of nitration.

[4] The control consisted of acetylated activator protein in 0.01 M Tris-HCl (pH 8.0) containing ethanol (1%).

TABLE II: Summary of Some Properties of the Tyrosine Residues of Activator Protein.

chemical property	Tyr residue 99	Tyr residue 138
nitration with tetranitromethane		
$+Ca^{2+}$	+[a]	+
$-Ca^{2+}$	+	−
acetylation with *N*-acetylimidazole		
$+Ca^{2+}$	+	−
$-Ca^{2+}$	+	+
pK in native protein		
$+Ca^{2+}$	10.1	11.9
$-Ca^{2+}$	10.4	11.9
pK after nitration		
$+Ca^{2+}$	7.3	8.6
$-Ca^{2+}$	7.3	8.1

[a] A "+" indicates reaction with reagent and a "−" indicates resistance to reaction with reagent.

Discussion

The results of chemical modification of the tyrosine residues of activator protein presented above, taken together with studies previously reported from this laboratory (Klee, 1977; Richman & Klee, 1978), have provided a basis for distinguishing the two tyrosine residues of the protein. Some of the chemical properties of these residues are summarized in Table II.

Tyrosine residue 99 behaves in all respects like an exposed tyrosine of a protein. It titrates with an apparent pK of 10.4 in the absence of Ca^{2+} and 10.1 in the presence of Ca^{2+} (Klee, 1977). It reacts with both *N*-acetylimidazole and tetranitromethane regardless of the presence or absence of Ca^{2+}. After nitration it exhibits an apparent pK of 7.2–7.4 which is not perturbed by Ca^{2+} binding (Richman & Klee, 1978).

Tyrosine residue 138, in contrast, is in a unique microenvironment, as reflected by its high apparent pK of 11.9 in the native protein and 8.1–8.6 after nitration. As a nitrotyrosine residue its apparent pK is increased by Ca^{2+} binding from 8.1 to 8.6, indicating that there is a dramatic change in the microenvironment of this residue upon Ca^{2+} binding. The lack of an effect of Ca^{2+} on the apparent pK of this tyrosine residue in the native protein may result from the high pH to which the protein is exposed during the titration. Consistent with the titration results is the decreased reactivity of this residue with *N*-acetylimidazole in the presence of Ca^{2+}. It appears that in the presence of Ca^{2+} tyrosine residue 138 becomes relatively inaccessible to solvent. However, this is the only condition under which it reacts with tetranitromethane. Tetranitromethane, which is a nonpolar compound, has been reported to react with "buried" tyrosine residues of other proteins (Glazer, 1976). The lack of reactivity of tyrosine residue 138 with tetranitromethane in the absence of Ca^{2+} is puzzling since the results (see Table II) indicate that it is more exposed to solvent under this condition. Perhaps the microenvironment of tyrosine residue 138 is sufficiently negatively charged and tightly hydrated in the absence of Ca^{2+} to make it inaccessible to non-

polar compounds like tetranitromethane. Binding of Ca^{2+} in this region may decrease its polarity and extent of hydration both by neutralizing the negative charges and by internalization of tyrosine residue 138. A more complete understanding of the microenvironment must await the determination of the three-dimensional structure of activator protein. It is clear from this and previous studies from this laboratory (Klee, 1977; Richman & Klee, 1978) that tyrosine residue 138 is in an unusual microenvironment that is substantially altered upon binding of Ca^{2+}.

Activator protein has been reported to resemble troponin C (Wang et al., 1975; Stevens et al., 1976; Watterson et al., 1976; Drabikowski et al., 1977; Walsh et al., 1977). The amino acid sequence of bovine brain activator protein (Vanaman et al., 1977) shows that this protein is highly homologous to both rabbit skeletal muscle troponin C (Collins et al., 1977) and bovine cardiac troponin C (Van Eerd & Takahashi, 1976). It is a member of a super-family of Ca^{2+} binding proteins that, in addition to the troponin C's, includes the alkali light chains of myosin and the parvalbumins (Barker & Dayhoff, 1976). From the crystal structure of parvalbumin, Kretsinger & Barry (1975) have identified putative Ca^{2+} binding residues in troponin C. This analysis has been extended by homology to activator protein by Vanaman et al. (1977), who places tyrosine residue 99 in the third Ca^{2+} binding loop and tyrosine residue 138 in the fourth. A free phenolic hydroxyl group is necessary for acetylation of tyrosine by *N*-acetylimidazole and also, indirectly, for reaction of phenols with tetranitromethane (Bruice et al., 1968). The results of the chemical modification studies (Table II) imply that if either tyrosine residue of activator protein is bound to Ca^{2+} through its phenolic hydroxyl group, such binding does not prevent reaction with *N*-acetylimidazole or tetranitromethane.

Reaction of activator protein with tetranitromethane does not lead to any change in the ability of the protein to activate cAMP phosphodiesterase (Walsh & Stevens, 1977; Richman & Klee, 1978). However, reaction with *N*-acetylimidazole decreases the apparent affinity of activator protein for phosphodiesterase. The results indicate that it was not acetylation of the tyrosine residues that was responsible for alteration of activity. Note that the preparation of activator protein that showed the greatest loss of affinity for the enzyme was the one reacted with *N*-acetylimidazole in the presence of Ca^{2+}, conditions in which the protein is presumably in its active conformation. Under these conditions only one of the two tyrosine residues (99) was acetylated. The preparation in which both tyrosine residues were acetylated had a much smaller loss in affinity for enzyme. In addition, after mild deacetylation of the tyrosine residues under conditions in which the lysine residues should remain acetylated, the affinity of these activator protein preparations for enzyme was not increased (Table I). It seems probable that acetylation of one or more of the lysine residues is responsible for the decreased affinity of activator protein for enzyme. The results also suggest that different lysine residues are acetylated by *N*-acetylimidazole in the presence of Ca^{2+} than in the presence of EGTA.

The results presented taken together with those previously reported from this laboratory (Richman & Klee, 1978) provide the basis for selectively nitrating either tyrosine residue 99 or tyrosine residue 138 of activator protein with little change in activity (activation of cAMP phosphodiesterase). Further studies on the interaction of these protein spectral probes with Ca^{2+} and other proteins are in progress. The results of these chemical modification reactions of activator protein have provided new insights into the separate microenvironments of the tyrosine residues.

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Light-Stimulated, Magnesium-Dependent ATPase in Toad Retinal Rod Outer Segments†

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ABSTRACT: Bleaching of rhodopsin increases adenosine and guanosine triphosphatase (ATPase and GTPase) activities present in the retinal rod outer segments (ROS) of the toad *Bufo marinus*. The light-stimulated activity travels with either broken or intact ROS widely separated on a metrizamide density gradient. The effect is primarily linked to the bleaching of rhodopsin in the red rods since the enzyme is half-stimulated by bleaching about 10% of the rhodopsin in either orange or blue light. The K_ms for ATP and GTP are 30 μM and 0.21 mM, respectively. Both activities appear to be catalyzed by the same enzyme since adenylyl imidodiphosphate (AMP-PNP) is a more effective inhibitor of both the ATPase and the GTPase than its guanyl analogue, GMP-PNP. The stimulation by bleaching is unaffected by the monovalent cations Na^+ or K^+ or by three different inhibitors of the mitochondrial ATPase. The light stimulation requires Mg^{2+}, and it is not seen when Ca^{2+} is the only divalent cation added. The chelating agent 2,2′-ethylenedioxybis(ethyliminodiacetic acid) increased the light stimulation still further, while adding Ca^{2+} lessened it. The activity was 0.9 ± 0.25 μmol of P_i released (mg of rhodopsin)$^{-1}$ h^{-1} and increased by a factor of 2.1 ± 0.3 in the light under optimal conditions. Low concentrations of Triton X-100 do not remove the light effect so it is not due to a membrane permeability change. The light stimulation is not minicked by the addition of *all-trans*-retinal. The fractional light activation which occurs when bleached and native membranes are mixed together is linear with the proportion of bleached membranes present.

The rod outer segments (ROS)[1] of the vertebrate retina can generate an electrical signal upon absorbing a single quantum of light (Yau et al., 1977). A sodium current flows into the outer segment of the rod cell in the dark and is inhibited in the light (Tomita, 1970; Hagins et al., 1970), changing the electrical potential across the plasma membrane. For dark-adapted vertebrate retinas half-maximal response of the rod requires about 30 quanta absorbed per rod (Fain & Dowling, 1973) or, in the case of the animal used in this study, the toad *Bufo marinus,* the bleaching of about one rhodopsin in 10^8 (Fain, 1975). Two possible mechanisms for amplifying the light signal to an electrical one have been advanced from the data published so far. One is that a phosphodiesterase is activated by the bleaching of rhodopsin and that the change in levels of 3′,5′-c-GMP in the ROS leads to a change in the sodium permeability of the outer segment plasma membrane (Woodruff et al., 1977). Another is that Ca^{2+} ions, sequestered in the flat discs whose membranes contain rhodopsin and which fill the ROS, are released into the outer segment cytoplasm when light bleaches rhodopsin and cause the sodium current to drop (Hagins and Yoshikami, 1974).

The theory that an ion might be released from the discs upon bleaching prompted an investigation to see what role an adenosine triphosphatase (ATPase) activity of the ROS might play in transporting ions across the disc membrane. The ionic gradients across ROS disc membranes in a living cell are not known (as opposed to those across the plasma membrane), although there is some evidence for a gradient of free Ca^{2+}. The Ca^{2+} content of freshly isolated ROS discs has been measured by Szuts & Cone (1977) and found to be about 0.1–0.2 Ca^{2+} ion per rhodopsin molecule or about 5 mM inside the disc, although most of this Ca^{2+} may be bound. In addition, the Ca^{2+} concentration in dark-adapted outer segment cytoplasm is about 1 μM since somewhat higher Ca^{2+} concentrations can affect the light response in the presence of the ionophore X537A (Hagins & Yoshikami, 1974). An ATP-dependent uptake of Ca^{2+} into bovine discs has also been reported (Schnetkamp et al., 1977) and may represent transport against a concentration gradient. None of the ATPase activities which are found in ROS preparations has been correlated with this process. In fact, it has been difficult to prove that any ATPase does come from the ROS. A Mg^{2+}-ATPase has been measured in bovine ROS preparations relatively free from mitochondrial contamination (Hendriks, 1975; Berman et al., 1977) but no activation by Ca^{2+} of this ATPase has been shown.

This work describes an ATPase in the ROS of the toad *Bufo marinus* which is activated as much as 2.5-fold by light (and

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[1] Abbreviations used: ROS, rod outer segment; EGTA, 2,2′-ethylenedioxybis(ethyliminodiacetic acid); EDTA, ethylenediaminetetraacetic acid; AMP-PNP, adenylylimidodiphosphate; GMP-PNP, guanylylimidodiphosphate; PK, pyruvate kinase; LDH, lactic dehydrogenase; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; PEP, phosphoenolpyruvate.

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