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UV-Induced Vanadate-Dependent Modification and Cleavage of Skeletal Myosin Subfragment 1 Heavy Chain. 2. Oxidation of Serine in the 23-kDa NH_2-Terminal Tryptic Peptide†

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ABSTRACT: Myosin subfragment 1 (S1) can be specifically photomodified at the active site without polypeptide chain cleavage by irradiating the stable MgADP–orthovanadate–S1 complex with UV light above 300 nm [Grammer, J. C., Cremo, C. R., & Yount, R. G. (1988) *Biochemistry* (preceding paper in this issue)]. Here, the UV spectral properties of photomodified S1 were used to determine the nature and location of the photomodified residue(s) within S1. By comparison of the unusual pH dependence of the UV absorption spectrum of the photomodified S1 to that of the S1–MgADP–V_i complex as a control, the photomodified residue(s) was (were) localized to the 23-kDa NH_2-terminal tryptic peptide of the heavy chain. $NaBH_4$ reduced the photomodified S1, but not the control, to regenerate the original spectral properties and ATPase activities of the unmodified S1. Amino acid analysis of photomodified S1 reduced with NaB^3H_4 gave only [^{3}H]serine, suggesting the hydroxyl group of serine had been oxidized to a "serine aldehyde". The pH dependence of the absorption spectrum of the photomodified enzyme can be explained by an equilibrium between a chromophoric enolate anion of the serine aldehyde (favored in base) and less chromophoric keto and enol forms (favored in acid). The oxidized serine(s) was (were) shown to be directly involved with the vanadate-dependent photocleavage of the S1 heavy chain previously described by Grammer et al. (1988). This serine(s) is (are) likely to be important to the binding and hydrolysis of the γ-PO_4 of ATP at the active site of S1.

In the previous paper (Grammer et al., 1988) we have shown that irradiation with UV light above 300 nm of the stable MgADP–orthovanadate (V_i)[1]–myosin subfragment 1 (S1) complex covalently modified the enzyme and rapidly released trapped MgADP and V_i from the active site. This photomodified S1 had Ca^{2+}ATPase activity 4–5-fold higher than that of nonirradiated control S1, while the K^+EDTA-ATPase activity was below 10% that of control. Analysis of the total number of thiols indicated that the photomodification did not involve SH1, SH2, or any other thiols. Irradiation of the

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[1] Abbreviations: serine aldehyde, form of serine in which the hydroxyl group has been oxidized to the level of aldehyde; V_i, orthovanadate; S1, myosin subfragment 1.

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S1–MgADP–V_i complex also increased the absorbance of the enzyme at 270 nm, which correlated with release of V_i from the active site. Although this suggested that an aromatic amino acid(s) may be involved, analysis by three different methods showed no loss of tryptophan.

Unlike irradiated dynein–MgADP–V_i complexes which undergo photoactivated polypeptide chain cleavage (Lee-Eiford et al., 1986), we found that no peptide bonds were cleaved in photomodified S1 (Grammer et al., 1988). However, we showed that if the photomodified S1–MgADP–V_i complex was formed and irradiated with UV light, the S1 heavy chain was specifically cleaved into two fragments (Grammer et al., 1988). Therefore, the photomodified S1 appears to be a stable intermediate in a multistep vanadate-dependent reaction which leads to cleavage of the polypeptide chain. If this is true, an understanding of the chemistry of this photomodification in S1 will be essential to determine a general mechanism of vanadate-dependent photochemical cleavage of polypeptides that may apply to other enzymes, such as dynein.

In this paper we describe the chemical nature of the photomodified enzyme. This was of special interest to us because it most likely involved an amino acid residue(s) at the polyphosphate binding site of myosin, a region of the active site that has not yet been labeled by any method. Previously, by analysis of difference UV spectra, we have shown (Grammer et al., 1988) that the new chromophoric residue had an extinction coefficient of 12000 M^{-1} cm^{-1} at 270 nm in denaturing buffer at pH 8.0. Because this change in extinction of the protein (S1 alone has an extinction coefficient at 280 nm of 86250 M^{-1} cm^{-1}; Wagner & Weeds, 1977) was so large, and was present under denaturing conditions, we used it as an assay to characterize the nature and location of the photomodified amino acid residue(s).

On the basis of the pH dependence of the absorption spectrum of photomodified S1 and its tryptic fragments, we have localized the photomodified residue to the 23-kDa heavy chain of S1. Reduction of the photomodified S1 with $NaBH_4$ appeared to regenerate native enzyme on the basis of the loss of the new chromophoric properties and on the basis of the recovery of normal ATPase activities. Amino acid analysis of photomodified S1 treated with NaB^3H_4 indicated that serine was the only radioactively labeled amino acid. An oxidized serine residue (serine aldehyde) was proposed as the photomodified amino acid to explain both the spectral properties and the reaction with $NaBH_4$. We also show that this serine residue is directly involved in the UV-induced vanadate-dependent cleavage of the photomodified S1 heavy chain. The oxidizable serine residue in the 23-kDa peptide may be critical for γ-PO_4 binding of ATP at the active site of S1.

Materials and Methods

The commercial compounds and their sources were as follows: $NaBH_4$ and Pronase type VI from *Streptomyces griseus* (Sigma); NaB^3H_4 (Dupont-New England Nuclear); arabinose (Eastman); SDS (Baker for electroelution and Pierce for electrophoresis). Enzyme preparations and assays were as described (Grammer et al., 1988). Absorbance spectra were recorded on a Varian 2200 spectrophotometer on line to a Varian DS-15 computer. Difference spectra were generated by storing the data for two spectra in the computer and subtracting them numerically. This method allowed the base line to be established with water and avoided the problems of base-line corrections with strongly absorbing samples. The total absorbances of samples at all wavelengths were adjusted to less than 1.2 absorbance units to avoid nonlinear response and the uncertainty in subtracting two large values.

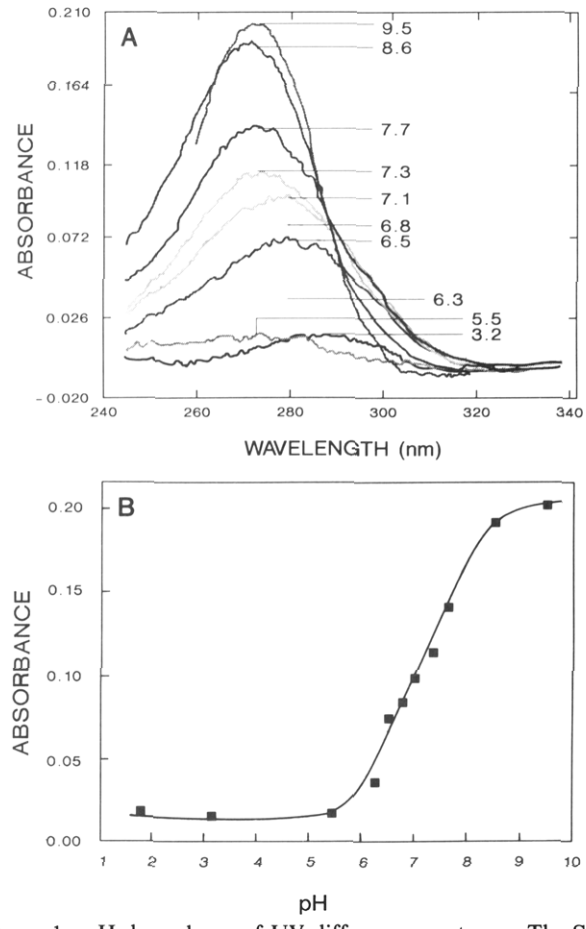

FIGURE 1: pH dependence of UV difference spectrum. The S1–MgADP–V_i complex and photomodified S1 were prepared at identical protein concentrations (4.0 mg/mL) as described under Materials and Methods. Aliquots (0.3 mL) were denatured by dilution into 0.4 mL of 7 M unbuffered guanidine hydrochloride prior to adding 0.1 mL of an appropriate buffer (500 mM stock concentration) to adjust the pH to the final values indicated. (A) Each spectrum is the difference between the spectrum of the photomodified sample and the spectrum of the S1–MgADP–V_i sample at the indicated pH. (B) The titration curve is a plot of the maximum absorbance at each pH value from the data in (A).

Results

We have previously shown (Grammer et al., 1988) that irradiation of the S1–MgADP–V_i complex caused a large increase in the absorbance of the enzyme at 270 nm. The absorbance increased over the same irradiation time course as changes in Ca^{2+}ATPase activity and the release of V_i and ADP from the active site, indicating that the new chromophoric properties were due to a modified residue at the active site. This spectral change was most clearly analyzed by measuring the UV difference spectrum between the photomodified enzyme and the S1–MgADP–V_i complex. If the samples were denatured in guanidine hydrochloride, the difference between the photomodified and the control (denatured complex) corresponded to a 15% increase in total absorbance of the control.

As a first step to identify the photomodified residue, we investigated the effect of pH on the UV difference spectrum. Figure 1A shows that the difference between the spectrum of the photomodified and the control sample was insignificant at pH 5.5 and below, but as the pH was increased to 9.5 the difference increased, and the wavelength maximum shifted from 281 to 272 nm. The spectrum at pH 9.5 could not be determined below 260 nm due to the large absorbances for both the photomodified and control samples below this

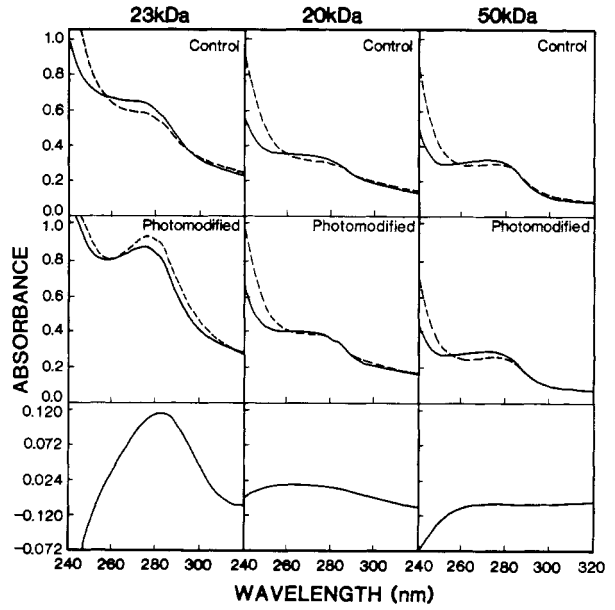

FIGURE 2: pH dependence of UV difference spectra of tryptic peptides of S1. The S1–MgADP–V_i complex and photomodified S1 were prepared at 4.0 mg/mL as described under Materials and Methods. Samples (8.8 mg) were then digested with trypsin (1/100 w/w) at 25 °C for 10 min, diluted with electrophoresis sample buffer, and boiled for 3 min prior to electrophoresis on a single-well, 3.0 mm thick, 14 × 10 cm, 12% gel according to Laemmli (1970). Peptides were visualized by soaking the gel on ice in 1 M KCl for 10 min. The 23, 50-, and 20-kDa tryptic peptides were excised and electroeluted in an IBI (New Haven, CT) Model UEA analytical electroelutor. The SDS concentration of the electroelution buffer was 0.01%, and bromophenol blue was omitted from the salt cushion to avoid interference with UV spectral measurements. Peptides were dialyzed against H_2O, lyophilyzed to dryness, resuspended in 8 M guanidine hydrochloride–50 mM Tris, pH 8.0, and filtered through a 0.4-μm filter. To adjust the pH for spectral measurements, samples were diluted with an equal volume of either S1 buffer (pH 7.0 final) or 100 mM HCl–KCl, pH 1.0, buffer (pH 3.0 final). In the six upper panels are the spectra at both pH 3.0 (—) and pH 7.0 (– –) for either the control (S1–MgADP–V_i complex sample) or the photomodified S1. The lower panel shows the difference for each tryptic peptide between the spectrum at pH 7.0 and the spectrum at pH 3.0, where the spectrum at pH 3.0 has been normalized to the base line to remove the effect of different protein concentrations between the samples [(photomodified S1 minus control at high pH) – (photomodified S1 minus control at low pH)].

wavelength (>1.5 absorbance units). The apparent equivalence point (pH = pK_a) was estimated to be between 7.0 and 7.5 on the basis of a plot of the maximum difference absorbance versus the pH (Figure 1B). The pH effect upon the magnitude and position of the difference spectrum was quickly and completely reversible (data not shown). Extensive digestion of the protein with Pronase (1/100 w/w additions at 0, 20, and 40 min and incubation overnight at 25 °C) had no effect upon any aspect of the difference spectrum, indicating that residual secondary structure and/or reversible interaction between distant residues in the sequence was probably not involved.

The photomodified residue(s) was (were) likely to be in the heavy chain as the light chains are not known to be directly involved with the active site in skeletal myosin (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). By examining the pH dependence of the difference spectra, it was possible to determine which tryptic fragment of the heavy chain was photomodified. After limited trypsin treatment, photomodified S1 and the S1–MgADP–V_i complex (as a control) were electrophoresed on SDS gels, and the characteristic 23-, 50-, and 20-kDa peptides of the heavy chains were excised and electroeluted. Figure 2 shows the pH dependence

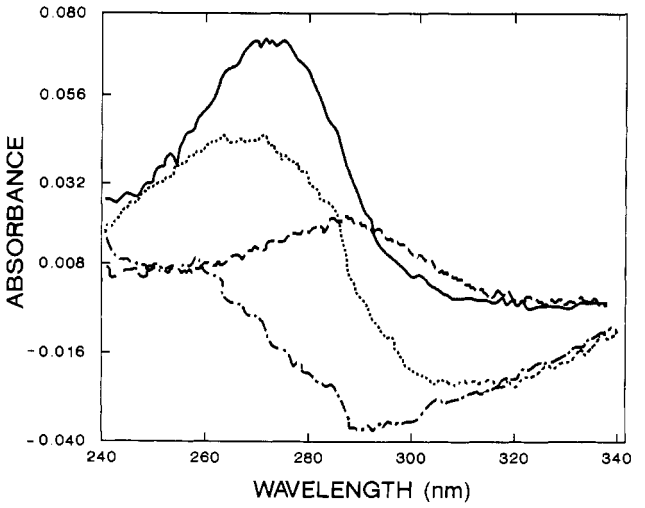

FIGURE 3: Effect of $NaBH_4$ upon UV difference spectrum. The S1–MgADP–V_i complex and photomodified S1 were prepared at 4.0 mg/mL as described under Materials and Methods. A 0.23-mL aliquot of each sample was diluted to a final volume of 1.0 mL with either S1 buffer or 7.5 M guanidine hydrochloride–50 mM Tris, pH 8.0, and a 40-fold excess of $NaBH_4$ (stock prepared in 0.1 N NaOH) over enzyme or an equivalent amount of 0.1 N NaOH was added. The absorption spectrum for each sample was collected after 1-h incubation on ice. Each spectrum is the difference between the spectrum of the photomodified sample and the spectrum of the S1–MgADP–V_i sample under the indicated conditions. Denatured in guanidine hydrochloride without (—) and with $NaBH_4$ (– –) or in S1 buffer without (···) and with $NaBH_4$ (–·–).

of the difference spectrum for each denatured tryptic peptide of the photomodified and control samples. The magnitudes of the absorbances of the different tryptic peptides cannot be compared because the concentrations of the peptides were not the same. Extinction coefficients could not be measured reliably in the presence of the considerable light scattering, presumably due to the presence of SDS micelles. However, the spectra at the two pH values within each panel can be directly compared in magnitude as they were the same sample and concentration. From the total spectra, the only peptide that appeared to show a significant difference between the control and photomodified samples was the 23-kDa peptide. At high pH the photomodified 23-kDa peptide had a unique spectral shape and was the only sample that showed a higher absorbance at high pH than at low pH. The normalized pH difference spectra in the bottom panels show that the 23-kDa peptide had a maximum difference at 280 nm, whereas neither the 20-kDa nor the 50-kDa peptides showed a significant difference. The difference spectrum maximum at 280 nm for the 23-kDa peptide at pH 7.0 was consistent with the position of the absorbance maximum of photomodified S1 at pH 7.0 in Figure 1. These results indicate that the spectral properties of the photomodified residue were specific to the 23-kDa peptide.

The S1–MgADP–V_i complex contains vanadium in its highest oxidation state (+5). Thus, it was likely that the effect of UV irradiation was to catalyze vanadate reduction, allowing the vanadate to accept electrons from an amino acid(s) at the active site, thus oxidizing the protein. This suggested that it might be possible to reduce photomodified S1 by treatment with $NaBH_4$. Figure 3 shows the effect of $NaBH_4$ upon the difference spectrum between the photomodified and control samples. In the absence of guanidine hydrochloride, the positive part of the difference spectrum, corresponding to the chromophoric residue(s) was signficantly decreased by $NaBH_4$ treatment. The negative part of the difference spectrum above 300 nm was not affected, as this aspect of the spectrum was

Table I: Effect of $NaBH_4$ upon ATPase Activities[a]

sample	% Ca^{2+}ATPase		% K^+EDTA-ATPase	
	$-NaBH_4$	$+NaBH_4$	$-NaBH_4$	$+NaBH_4$
control (photolyzed)	100	100	100	100
S1–MgADP–V_i complex	7.6 (0.9)	5.1 (0.7)	1.1 (0.3)	1.4 (0.1)
photomodified S1	369 (5)	125 (7)	5.7 (0.3)	61 (4)

[a] The S1–MgADP–V_i complex and photomodified S1 were prepared as described under Materials and Methods. The photolyzed control was treated in an identical manner except that V_i was not added. Irradiation had no effect upon control ATPase activities. Samples were reduced for 30 min on ice by adding a 4-fold excess of $NaBH_4$ over enzyme from a concentrated stock made in 0.1 N NaOH. An equivalent amount of 0.1 N NaOH was added to the unreduced samples. A 20-fold excess of arabinose over enzyme was added to react with excess $NaBH_4$ before assaying for ATPase activities. Each datum is expressed as the mean and standard deviation from the results of three independent experiments.

shown by Grammer et al. (1988) to be due only to the presence of the MgADP–V_i complex. In the presence of guanidine hydrochloride, the difference spectrum had a maximum absorbance at 270 nm which was decreased and red-shifted to 285 nm after $NaBH_4$ treatment. $NaBH_4$ had no effect upon the total spectra of the control samples (data not shown). On the basis of these spectral data, $NaBH_4$ appeared to react with the photomodified enzyme (but not the control) to generate a species which was nearly identical with the original S1–MgADP–V_i complex or the denatured complex, respectively. A unique conformation of the enzyme was not necessary for $NaBH_4$ to react with the photomodified residue(s), as the spectral changes were observed for both native and denatured samples.

To confirm that $NaBH_4$ was reacting with the photomodified residue(s) to regenerate unmodified S1, as was suggested from the spectral measurements (Figure 3), we examined the effects of $NaBH_4$ upon the ATPase activities of control S1 (irradiated), the S1–MgADP–V_i complex, and photomodified S1 (Table I). $NaBH_4$ had no effect upon either the Ca^{2+}- or the K^+EDTA-ATPase activities of irradiated control S1. This result was expected as myosin does not contain functional groups such as aldehydes, ketones, and disulfides that can be reduced with $NaBH_4$. The S1–MgADP–V_i complex had low Ca^{2+}- and K^+EDTA-ATPase activities that were not affected by $NaBH_4$ treatment, indicating that $NaBH_4$ did not destabilize the trapped nucleotide at the active site. The photomodified enzyme had the characteristic elevated Ca^{2+}ATPase activity and low K^+EDTA-ATPase activity previously described by Grammer et al. (1988). In contrast to the control S1 and the S1–MgADP–V_i complex, $NaBH_4$ treatment of photomodified S1 caused a significant drop in the elevated Ca^{2+}ATPase activity from 369% to 125% of the control level. Similarily, $NaBH_4$ caused the inactivated K^+EDTA-ATPase of the photomodified enzyme (5.7% of control) to increase to 61% of the control level. These results would be expected if the effect of $NaBH_4$ was to return the photomodified enzyme back to a native form. However, under these reaction conditions the photomodified residue(s) was (were) apparently not stoichiometrically reduced because $NaBH_4$ treatment did not fully return the Ca^{2+}- or the K^+EDTA-ATPase activities to control levels (see below).

Once it had been established that $NaBH_4$ reacted only with the photomodified S1 and did not react with the control samples (Figure 3; Table I), NaB^3H_4 was used to specifically radiolabel the photomodified residue. The time course of reduction indicated that the reaction was complete after 30 min on ice. Control S1 incorporated <5% of the tritium incorporated into photomodified S1 as measured by tritium in trichloroacetic acid precipitable protein (data not shown). The NaB^3H_4-treated photomodified S1 was then hydrolyzed and submitted for amino acid analysis to characterize the labeled residue (Figure 4). Essentially all of the injected radioactivity eluted in fractions 20 and 21 which contained the entire serine peak and the tail of the threonine peak as determined from the positions of amino acid standards. No detectable levels of any other amino acids eluted in these two fractions. Although the radioactivity appeared to coelute with serine, indicating that serine and not threonine was labeled, the specific activities for both serine and threonine were calculated to ensure that threonine was not labeled. To do this, aliquots of fractions 20 and 21 were independently reinjected with the ninhydrin coil in line to quantitate the amino acids by comparison with an amino acid standard. As expected, the specific activity for threonine was not constant (286 and 1211 cpm/nmol for fractions 20 and 21, respectively), indicating that it was not radioactive. In contrast, the specific activity of serine was the same for both fractions (662 and 660 cpm/nmol for fractions 20 and 21, respectively), indicating that only serine was radioactively labeled. The specific activity of the photomodified S1 after reduction with NaB^3H_4 [848 ± 80 cpm/nmol of serine; assuming 49 serine/S1; M. Elzinga (personal communication) and Frank and Weeds (1974)] was slightly reduced to 667 ± 50 cpm/nmol of serine after hydrolysis. However, this is the same specific activity calculated for the purified serine peak (see above), indicating that all of the radioactivity in the hydrolysate was [^{3}H]serine. These results suggest the photomodified S1 contains a serine aldehyde which on reduction with NaB^3H_4 yields a serine residue labeled with tritium on the β-carbon.

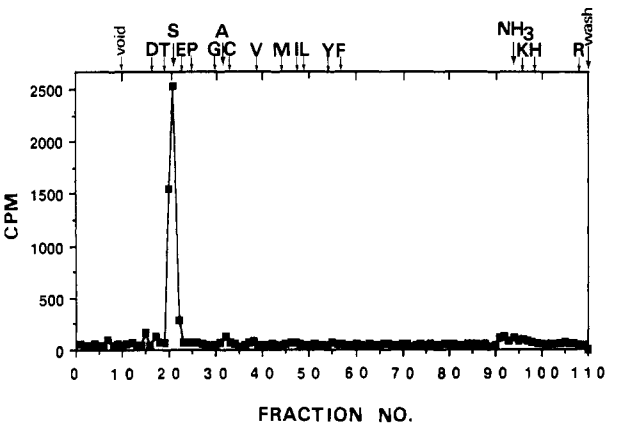

FIGURE 4: Amino acid analysis of NaB^3H_4-reduced photomodified S1. Photomodified S1 was prepared as described under Materials and Methods in S1 buffer and reduced with a 4-fold excess of NaB^3H_4 over enzyme for 30 min on ice. The reduction was quenched with a 40-fold excess of arabinose over enzyme for 30 min on ice. After purification by centrifugation through a Sephadex G-50 fine column, the sample was dialyzed against 0.1% trifluoroacetic acid, lyophilized, and hydrolyzed in 6 N HCl for 22 h at 110 °C. A 1.0-nmol sample was analyzed on a Beckman Model 121 MB amino acid analyzer, with the ninhydrin coil bypassed, using a gradient system designed for high resolution of all amino acids except tryptophan and histidine. One-minute fractions were collected, and aliquots were analyzed for radioactivity. The letters above indicate the elution times (corrected to account for the delay between the ninhydrin detector and the fraction collector) for a set of amino acid standards.

Discussion

The purpose of this study was to establish the chemical nature of the large increase in absorbance observed during the vanadate-promoted photomodification of S1 (Grammer et al.,

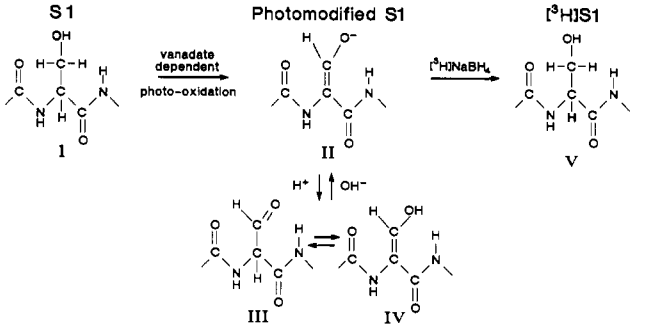

FIGURE 5: Chemical reaction scheme for the vanadate-promoted photooxidation of serine.

1988) and, if possible, to locate the modified residue(s). A serine in the 23-kDa NH_2-terminal tryptic peptide of the heavy chain appears to be the modified residue. We propose the scheme in Figure 5 to explain the described spectral properties of the photomodified S1 and the effects of reduction with $NaBH_4$. Unmodified S1 (I) contains a serine in the 23-kDa tryptic peptide that is involved in some manner with the S1–MgADP–V_i complex. Photmodified S1 (II, III, and IV) is formed by irradiating the S1–MgADP–V_i complex with UV light in the 300–400-nm range to promote a two-electron oxidation of the serine residue to a "serine aldehyde".[2] The serine aldehyde, at higher pH values, will be mostly in the chromophoric enolate anion form (II), whereas in acid the equilibrium will shift to the comparatively nonchromophoric protonated forms III and IV. The serine aldehyde of the photomodified S1 (II, III, IV) can be reduced with NaB^3H_4 to [^{3}H]serine (V), where the tritium is stably incorporated on the β-carbon.

There were two key elements of our results which led to Figure 5. First, the photomodified enzyme contained a chromophore in the 23-kDa tryptic peptide of S1 whose absorbance was pH dependent (Figures 1 and 2). The chromophore absorbed more strongly between 270 and 280 nm under basic conditions than in acid. The pH dependence was reversible, as would be predicted for a simple protonation–deprotonation of a functional group. On the basis of this assumption, the plot of the absorbance versus pH (Figure 1B) indicated that the chromophoric group behaved like a weak acid with an apparent pK_a of 7.0–7.5. This finding is consistent with Figure 5, which involves a simple equilibrium between the deprotonated (II) and protonated (III, IV) forms of an oxidized serine.

The keto (III) and enol (IV) forms, in equilibrium under acidic conditions, are not expected to have large extinction coefficients in the 250–300-nm range. The keto form (III) contains the isolated carboxamide group which absorbs significantly only in the vacuum ultraviolet and an isolated aldehyde group which is expected to have an extinction coefficient of <100 M^{-1} cm^{-1} at 290 nm (Scott, 1964). In contrast, the enolate anion (II) favored under basic conditions is predicted to have a relatively large extinction coefficient in the 250–300-nm region by comparison with the spectral properties of structurally similar amidomalonaldehyde derivatives. As an example, HCONHC(CHO)CH(OH) under acidic conditions, where the dissociation of the enolic proton is suppressed, has a maximum absorbance at 251 nm (ϵ = 15 100 M^{-1} cm^{-1}). However, in 0.01 N NaOH there is a bathochromic shift to 267 nm, with an ~2-fold increase in the extinction coefficient (ϵ = 29 700 M^{-1} cm^{-1}) (Samek et al., 1977). This general trend involving a bathochromic shift accompanied by an increased extinction coefficient upon forming an enolate anion in base from a keto–enol tautomer in acid is not peculiar to amidomalonaldehydes but is also a general phenomenon of β-diketones ($H_3COCH_2COCH_3$) [see, e.g., Scott (1964)]. In fact, a shift in absorbance maximum to longer wavelengths generally accompanies oxyanion formation from a neutral precursor (Fox, 1979). The apparent pK_a estimated in Figure 1B of 7.0–7.5 for the photomodified S1 chromophore is a value in general agreement with pK_a values measured for structurally similar organic compounds. For example, by UV spectroscopic methods, the pK_a for the reaction of OH^- with the malonaldehyde derivative HCONHC(CHO)CH(OH) to give the enolate anion was found to be 3.46 (Samek et al., 1977). However, as expected, the peptide containing the serine aldehyde appears to be a weaker acid than this model compound possibly due to the partial negative charge of the amide carbonyl.

The second key element of our results was that the photomodified residue in the 23-kDa tryptic fragment of photomodified S1 could be reduced with $NaBH_4$. The large difference between the spectrum of photomodified S1 and that of the control under basic conditions could be diminished by $NaBH_4$ treatment (Figure 3). In addition, $NaBH_4$ treatment caused the ATPase activities of photomodified S1 to return to near control levels while having no effect upon control samples (Table I). An amino acid analysis of the photomodified enzyme that had been treated with NaB^3H_4 showed that the only radioactive product was [^{3}H]serine (Figure 4). These three results are consistent with Figure 5 in that reduction with $NaBH_4$ returns the enzyme back to an unmodified form by reacting with the chemical species that was responsible for the chromophoric properties of photomodified S1.

From these results we cannot determine the number of serines that may be involved in the photomodification. However, it seems likely that this is a specific modification of only one serine that is involved with complexing V_i at the ATP binding site. Also, our results do not rule out other modifications of the enzyme that depend upon photoactivation of the S1–MgADP–V_i complex. However, if other amino acids were modified, they did not greatly alter the UV absorption spectrum or the ATPase activities of S1 because reduction of the serine aldehyde with $NaBH_4$ appeared to return these properties of the enzyme back to near normal. They also did not involve thiols or tryptophan residues (Grammer et al., 1988).

It can be predicted from Figure 5 that reduction of the chromophore (II, III, IV) should not depend upon the conformation of the protein. As predicted, $NaBH_4$ was shown to alter the UV difference spectrum even under denaturing conditions (Figure 3), indicating that $NaBH_4$ could react regardless of the conformation of the protein. An alternative scheme that is also consistent with this result could involve a pH-dependent reversible association between two functional groups close together in the amino acid sequence. An example of this would be reversible Schiff base formation between an aldehyde and a lysine (Means & Feeney, 1968) within the 23-kDa peptide. This explanation is unlikely considering that exhaustive Pronase treatment did not alter the reversible pH-dependent properties of the chromophore. Furthermore, a Schiff base within the 23-kDa peptide becomes untenable as reduction with NaB^3H_4 would be expected to reduce the Schiff base to an acid-stable radioactive amine adduct which would have been easily distinguished from [^{3}H]serine in the amino acid analysis.

[2] The detailed mechanism of this vanadate-catalyzed photooxidation is not known and is a topic for further investigation.

It seemed reasonable to suspect that the serine aldehyde was involved in photocleavage of the photomodified S1–MgADP–V_i complex (Grammer et al., 1988). From the results in Figure 2, the photomodified residue, serine aldehyde, was found to be within the 23-kDa tryptic fragment of the heavy chain. In addition, irradiation of MgADP and V_i trapped on the photomodified S1 cleaves the heavy chain within the 23-kDa NH_2-terminal tryptic peptide. No cleavage of the heavy chain was observed if the photomodified S1 was reduced with $NaBH_4$ prior to the formation of a new MgADP–V_i complex and a second irradiation (data not shown). Therefore, this demonstrated conclusively that the serine aldehyde was necessary for the photocleavage reaction. To summarize, the effect of reduction on the photomodified enzyme was to return the absorption spectrum (Figure 3) and the ATPase activities (Table I) to near control levels and to prevent the photocleavage reaction that was characteristic of the unreduced photomodified complex.

It is not unexpected that serine should be involved with V_i complexation at the active site of S1. Vanadate spontaneously esterifies hydroxyl groups such as ethanol (Gresser & Tracey, 1985). A ligand exchange reaction or addition of a fifth ligand involving a serine ester with V_i at the active site may be the molecular basis of the slow isomerization from a reversible ternary complex (myosin–ADP–V_i) to a stable complex (myosin*–ADP–V_i) proposed from the kinetic results of Goodno (1979). Vanadium (V) is known to oxidize organic carbonyl compounds in the presence of light of >300 nm (Panwar & Guar, 1967) and to undergo reduction to vanadyl (IV) in the process (Satapathy et al., 1963). It is not known why irradiation caused vanadate to be released from the active site, but it is known that vanadium is one of the few transition metals that changes charge upon reduction; in the +5 oxidation state monomeric vanadate is negatively charged (VO_4^{3-} and various levels of protonation) whereas in the +4 state it is positive (VO^{2+}). After the protein oxidation, VO^{2+} would be unstable in the presence of oxygen at pH 8.0 and would be oxidized to vanadate (North & Post, 1984).

In conclusion, this paper describes the UV spectral properties of S1 that has been modified by UV light in the presence of vanadate. Using $NaBH_4$ treatment of the enzyme, we have shown that these spectral properties can be directly correlated to changes in ATPase activities, indicating that both phenomena were due to the same chemical species. The photomodified residue was a serine in the 23-kDa tryptic peptide of S1 which is likely to be involved in binding the γ-PO_4 of ATP at the active site. An oxidized form of this serine appeared to be involved in the vanadate-dependent photochemical cleavage of the S1 heavy chain. The location of photocleavage was approximately 21 kDa from the NH_2-terminal end of the heavy chain (Grammer et al., 1988). This is near the consensus GESGAGKT nucleotide binding sequence (Tong & Elzinga, 1983; Walker et al., 1982; Fry et al., 1986), suggesting perhaps that the serine in this sequence is the residue modified. Work is in progress to determine the exact location of the oxidized serine or serines. A reaction sequence (Figure 5) consistent with all the results involves oxidation of the serine to a serine aldehyde when UV light activates the S1–MgADP–V_i complex. This serine aldehyde can then be reduced back to serine with $NaBH_4$. The photomodified S1 containing the serine aldehyde is a stable intermediate which can be further oxidized in a vanadate-dependent manner to cleave the polypeptide chain (Grammer et al., 1988). This mechanism is likely to be involved in other ATPases such as dynein which can be specifically cleaved in the presence of vanadate and UV light (Lee-Eiford et al., 1986; Gibbons et al., 1987). The distinctive spectral changes that accompany these reactions in S1 may be useful to assay for oxidized serine residues in general or in other systems which involve enzyme–V_i complexes that do not undergo polypeptide chain cleavage upon UV irradiation. Most importantly, we show that reduction with NaB^3H_4 provides a way to radiolabel the protein. This approach will be essential to determine the location of the modified residues within the primary structure and provides a potential new method to localize phosphate binding sites on enzymes.

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Registry No. V_i, 14333-18-7; ATPase, 9000-83-3; serine, 56-45-1.

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