Interaction of Cibacron Blue F_3GA with Glutamine Synthetase: Use of the Dye as a Conformational Probe. 2. Studies Using Isolated Dye Fractions†

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Received July 2, 1984

ABSTRACT: By means of column chromatography on silicic acid, commercial preparations of Cibacron Blue F_3GA have been resolved into four major subfractions (fractions I–IV). The difference spectrum between free dye and dye bound to any given form of *Escherichia coli* glutamine synthetase (GS) is different for each dye fraction. Moreover, uniquely different spectral perturbations are associated with the binding of any one dye fraction to the taut, relaxed, dissociated, or oxidized forms of GS. On the basis of the magnitude of the differences in the difference spectra between free dye and the dye–GS complexes, fraction II is most suitable for monitoring the interconversion of the relaxed and taut forms of GS. Fraction II can also be used to measure the fraction of oxidized (inactive) GS that is present in apparently homogeneous GS preparations. In contrast to the other three fractions, the difference spectrum obtained immediately following the binding of fraction I to GS undergoes a time-dependent change which is associated with the covalent attachment of the dye to the enzymes. Fractions II, III, and IV apparently bind to the nucleotide binding site on GS because the difference spectrum obtained with these fractions can be quenched by the subsequent addition of 1–2 mM ADP. The primary but not the secondary complex formed between GS and fraction I can also be destroyed by ADP.

We showed previously that the spectrum of a commercial preparation of Cibacron Blue F_3GA is altered when it is bound to the glutamine synthetase (GS) from *Escherichia coli*. Because the spectral perturbation varies with the conformational state, the dye can be used to monitor conversion of the enzyme between relaxed (native-free), taut (native enzyme), and dissociated forms of the enzyme (Shapiro & Ginsburg, 1968; Kingdon et al., 1968) as well as inactivation of the enzyme by a mixed-function oxidation reaction (Levine et al., 1981; Oliver et al., 1982). While this work was in progress [see Federici & Stadtman (1979)], Weber et al. (1979) reported that commercial preparations of Cibacron Blue F_3GA are mixtures of several molecular species. They demonstrated that two components which were purified to apparent homogeneity exhibited very different protein binding characteristics. In the present report, we confirm that the commercial dyes are highly heterogeneous and describe the isolation of four chromatographically distinct major subfractions of dye from a single commercial sample. We show that the binding of each of these fractions to native glutamine synthetase elicits a uniquely different spectral perturbation and that there is considerable variability in the abilities of these fractions to discriminate between various conformational states of the glutamine synthetase.

Materials and Methods

Adenosine 5′-diphosphate (sodium salt) was from Sigma Chemical Co., St. Louis, MO. *n*-Butyl alcohol, ethyl acetate, *tert*-butyl alcohol, methyl ethyl ketone, and tetrahydrofuran were all reagent grade from Baker Chemicals. Preparations of native GS and GS that had been oxidized by the ascorbate–Fe^{3+}–O_2 oxidation system (Levine, 1983) were kindly provided by Dr. R. Levine. Analytical linear high-performance silica thin-layer chromatography (TLC) plates were obtained from Whatman-Reeve Angel, Inc. Silica gel 60 (0.2–0.5 mM, 35–70 mesh) for column chromatography was obtained from Brinkmann Instruments, Inc.

Thin-Layer Chromatography. Chromatography of the commercial Cibacron Blue F_3GA and purified subfractions was carried out on linear HP-K silica plates using either 4:3:2:1 (v/v) *tert*-butyl alcohol:methyl ethyl ketone:H_2O:NH_4OH as a solvent or [as described by Weber et al. (1979)] 48:7 (v/v) tetrahydrofuran:H_2O as solvent. All other reagents, enzyme preparations, materials, and methods were as described previously (Federici et al., 1985).

Results

Fractionation of Commercial Cibacron Blue F_3GA by Column Chromatography. The heterogeneity of a commercial preparation of Cibacron Blue was disclosed by thin-layer chromatography (Figure 1). Partial fractionation of the major components was achieved as follows: Cibracon Blue F_3GA from Ciba-Geigy (300 mg) was dissolved in 8 mL of H_2O and acidified with 2 mL of 1 M $NaHSO_4$. The solution was successively extracted 8 times with 10-mL portions of H_2O-saturated 1-butanol. The extracts were pooled, and ethyl acetate was added to give a butanol extract:ethyl acetate ratio of 4:1. This was added immediately to a silica gel column (2.5 × 44 cm) which had been equilibrated by washing with 2 bed volumes of a 4:1 butanol:ethyl acetate mixture. The column was eluted first with 1 L of 4:1 ethyl acetate:butanol and then with 3.5 L of 2.7:1 ethyl acetate:butanol. Fractions (200 mL) were collected. To concentrate the dye, each fraction was transferred to a separating funnel, and 2 mL of H_2O and 20 mL of cyclohexane were added. After gentle shaking, an immiscible mixture was formed which upon standing separated quickly into two clear layers; the dye was almost quantitatively recovered in the small aqueous layer (2–3 mL). The aqueous

† Supported by National Research Service Award 5F32 GMO 6338-02 to M.M.F.
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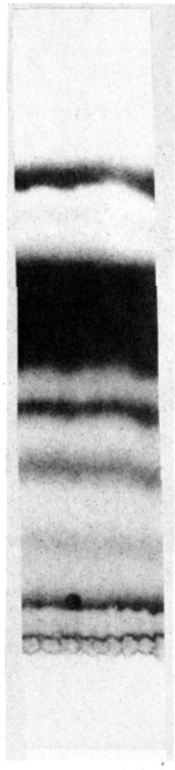

FIGURE 1: Heterogeneity of Cibacron Blue F_3GA. A solution of dye (0.18 mL, 1.51 mM) was applied in a streak to a preparative silica gel plate (2000 μm) and developed in an ascending direction in 40:30:20:10 (v/v) *tert*-butyl alcohol:methyl ethyl ketone:H_2O:NH_4OH.

layer from each fraction was subjected to TLC as described above, using the 48:7 tetrahydrofuran:H_2O solvent system. Those fractions which upon chromatography yielded a single band of dye of the same R_f were pooled. With this procedure, the commercial dye samples were resolved into four major blue subfractions and a number of minor fractions with colors ranging from greenish blue and bright blue to yellow and pink. The chromatographic behavior of the four major blue fractions is shown in Figure 2. They are numbered in the order of their elution from the silica column. Fraction I (R_f = 0.83) was eluted with the 4:1 ethyl acetate:butanol solvent system; all others, fractions II (R_f = 0.74), III (R_f = 0.69), and IV (R_f = 0.65), were eluted with the 2.7:1 ethyl acetate:butanol solvent. Whereas all four dye types were present in all commercial samples tested, a significant amount of fraction II was present only in a preparation obtained from Pierce Chemical Co. Therefore, the Pierce product was used for the isolation of fraction II. Each of the four pooled fractions was lyophilized and stored at −80 °C. Fresh solutions were prepared for each experiment.

Effects of GS Relaxation and Dissociation on the Difference Spectra Obtained with Various Dye Fractions. The absolute absorption spectrum of each one of the four dye fractions is very similar to that of an unfractionated dye preparation. However, as shown in Figure 3, the difference spectrum between free dye and dye bound to either taut, relaxed, or dissociated forms of GS is uniquely different for each dye fraction. Figure 3 shows also that fraction II is most suitable for monitoring the interconversion of taut and relaxed configurations.

Comparison of the Dye Complexes Obtained with Native and Oxidized Forms of GS. The difference spectrum between unfractionated dye and unfractionated dye bound to native GS is significantly different from that between dye and dye bound to GS that has been inactivated by mixed-function oxidation reactions (Federici et al., 1985). Even greater differences between native and oxidized GS were obtained with fractions I and II. As shown in Figure 4, the spectral difference between fraction I complexes of native and oxidized GS undergoes a slow time-dependent change. We show in the following section that this time-dependent change is due to a secondary reaction in which the dye becomes covalently bound to the enzyme.

In contrast, there is no time-dependent change in the difference spectra obtained following the initial binding of fraction

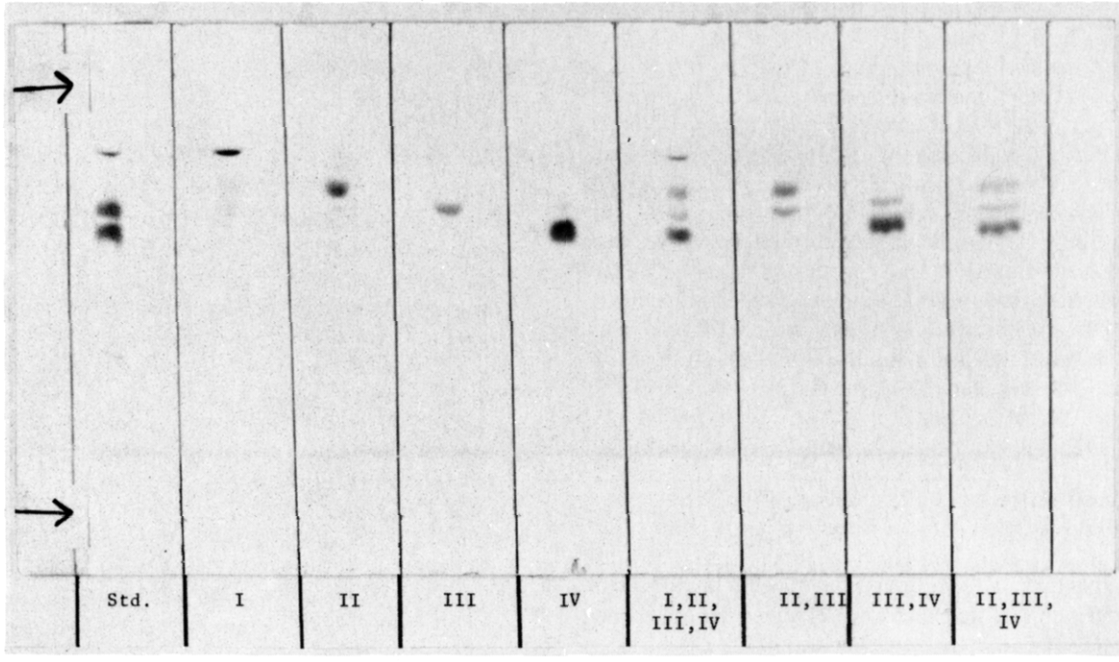

FIGURE 2: Thin-layer chromatography of dye fractions on linear HP-K analytical silica plates. The origin and solvent front are marked by the arrows. The solvent system used was 48:7 (v/v) tetrahydrofuran:H_2O. The designation for each channel is as follows: std. = 7 μL, 1.42 mM unfractionated Cibacron Blue F_3GA; I = 7 μL, 1.35 mM fraction I, R_f = 0.83 ± 0.03; II = 5 μL, 0.48 mM fraction II, R_f = 0.74 ± 0.02; III = 7 μL, 0.224 mM fraction III, R_f = 0.69 ± 0.02; IV = 5 μL, 1.32 mM fraction IV, R_f = 0.65 ± 0.02; the remainder of the channels represent equal volume mixtures of the appropriate solutions noted above; 7.0 μL of each mixture was applied at the origin.

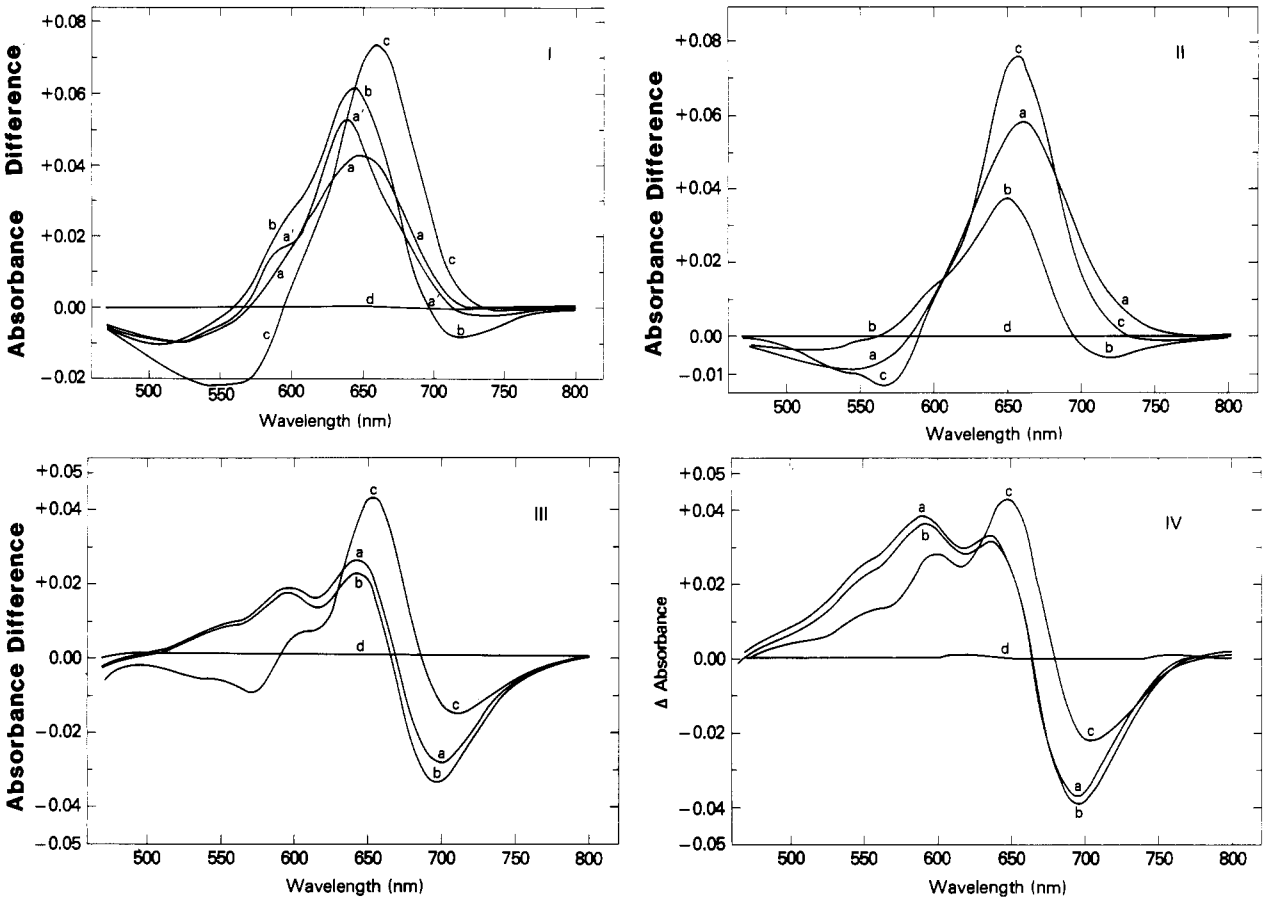

FIGURE 3: Difference spectra between each dye fraction alone and the fractions in the presence of different structural forms of unadenylylated GS. The base lines (curve d) were obtained for the standard reaction mixtures [80 μM dye, 100 mM KCl, 1 mM $MnCl_2$, and 35 mM Hepes (pH 7.5), ~25 °C] in both the sample and reference cuvettes by using automatic base-line correction of the Varian 219 spectrophotometer. The difference spectrum for the native taut form of GS is produced by the addition of 0.05 mL of a 15–20 mg/mL stock protein solution in the sample and final dialysate to the reference, respectively (curve a, zero time; curve a', after 6 h). The difference spectra for the relaxed and dissociated forms of GS are produced by the addition of 0.015 mL of 0.1 M NaEDTA (curve b, relaxed) followed by the addition of 0.02 mL of Tris/KOH [1.0 mL of 1 M Tris (pH 8.0) + 1.0 mL of 4.0 N KOH] (curve c, dissociated final pH 8.5–9.5) to both the sample and reference cuvettes, respectively; I = fraction I, R_f = 0.83 ± 0.03; II = fraction II, R_f = 0.74 ± 0.02; III = fraction III, R_f = 0.69 ± 0.02; IV = fraction IV, R_f = 0.65 ± 0.02. R_f refers to migration on silica in tetrahydrofuran/H_2O (48:7 v/v).

II to native or oxidized GS. With fraction II, the amplitude of the difference spectrum between the native GS–dye complex and the fully oxidized GS–dye complex is about twice as great as that obtained between the dye complexes of native GS and 50% oxidized GS. This suggests that fraction II may be useful in determining the fraction of oxidized (inactive) GS present in purified preparations of GS.

From direct binding measurements by the sedimentation technique (Federici et al., 1985), it was found that in the presence of a large excess of dye, approximately 1 equiv (0.85–0.9) of either fraction I or fraction II can be bound to each subunit of GS.

Irreversible Binding of Fraction I to GS. With either fraction II, III, or IV, the amplitude of the difference spectrum between dye vs. dye + GS reaches a maximum within 2 min and remains unchanged for several hours (data not shown). In contrast, the difference spectrum obtained immediately after fraction I is mixed with GS exhibits a single maximum at 666 nm and a minimum at 570 nm. However, with continued incubation for up to 6 h, there is a progressive change in the spectrum to a form exhibiting maxima at 638 and 590 nm (Figure 6). The occurrence of an isosbestic point at 645 nm suggests that the time-dependent change reflects a chemical process in which the primary GS–dye (I) complex is converted to a single uniquely different form. That this conversion involves covalent interaction of the dye with a functional group(s) on the enzyme is supported by the following facts: (1) Immediately after fraction I is mixed with GS, the dye can be displaced from the GS–dye complex by ADP (Figure 5), whereas it cannot be displaced from the secondary complex which forms with prolonged incubation (data not shown). (2) After the time-dependent change is complete (6 h, 30 °C), fraction I cannot be removed from GS by dialysis either for 3 days against 35 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.5) and 1 mM $MnCl_2$ or for 4 days against the same buffer containing 6 M guanidine hydrochloride. In control experiments, the dye in mixtures containing GS plus either unfractionated dye or fraction III or in a solution of fraction I alone (no GS) was almost completely removed by dialysis. (3) The dye remained bound to GS during electrophoresis on polyacrylamide gels (10%) in the presence of 0.1% sodium dodecyl sulfate. (4) Precipitation of the GS with trichloroacetic acid did not cause dissociation of the GS–dye (I) complex.

Quenching the Difference Spectrum with ADP. If ADP–Mn (1–2 mM) is added to both cuvettes immediately after the dye vs. dye + GS difference spectrum is recorded, the spectral difference is largely quenched (~85%), indicating that ADP can displace the dye from the GS–dye complex. This is illustrated in Figure 5 which shows the results obtained with fraction I. Similar results were obtained with any of the other three dye fractions and are typical of results obtained by others

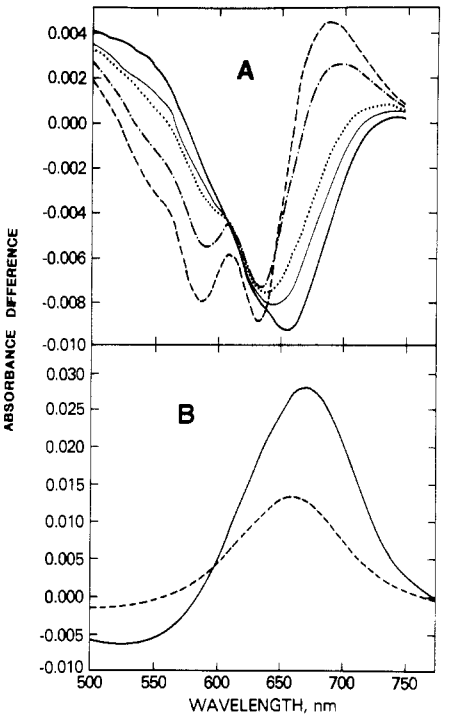

FIGURE 4: Difference spectra between dye bound to native GS and dye bound to oxidized GS. (A) Initially, both cuvettes contained 60 μM fraction I, 30 mM Hepes (pH 7.4), and 1.0 mM $MnCl_2$. After the base line was recorded, 30 μM native GS (subunit equivalents) was added to the sample cuvette, and 30 μM fully oxidized GS (i.e., completely inactivated GS) was added to the reference cuvette. The difference spectrum was recorded immediately (thick solid line) and after 10 (thin solid line), 30 (···), 75 (−·−), and 240 (- - -) min. (B) Initially, both cuvettes contained 60 μM fraction II, 30 mM Hepes (pH 7.4), and 1.0 mM $MnCl_2$. After the base line was recorded, 30 μM GS was added to the sample cuvette, and 30 μM fully oxidized GS was added to the reference cuvette. The difference spectrum (—) was recorded immediately but did not change even after 30 min. In an otherwise identical experiment (- - -), 30 μM GS which was only 50% oxidized (inactivated) was added to the reference cuvette.

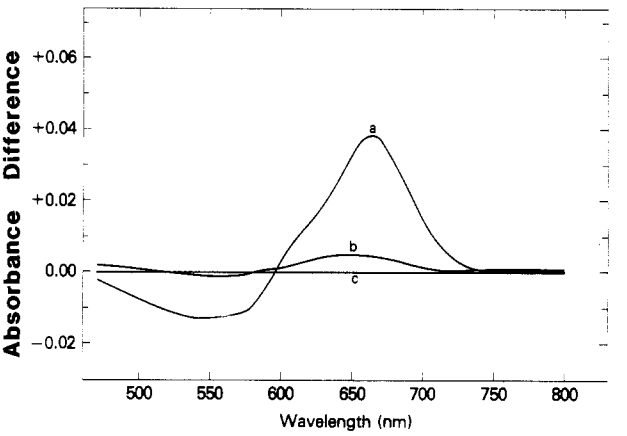

FIGURE 5: Quenching of the dye protein difference spectrum by ADP. The base line (curve c) was obtained for the dye reaction mixture [15.0 μM fraction I (R_f = 0.83 ± 0.03), 100 mM KCl, 1 mM $MnCl_2$, and 35 mM Hepes (pH 7.5), ~25 °C] in both the sample and reference cuvettes by using the automatic base-line correction of the Varian 219 spectrophotometer. The difference spectrum for the native taut form of $GS_{\bar{1}}$ (curve a) is produced by the addition of 0.05 mL of 15–20 mg/mL protein stock solution to the sample and final dialysate to the reference, respectively. The difference spectrum is quenched by the immediate addition of 3.4 mM ADP (curve b) to both sample and reference cuvettes. However, if the time-dependent reaction described in Figure 8 is allowed to occur, no displacement of the difference spectrum (using fraction I) occurs.

who have studied the binding of unfractionated dye preparations with various kinases, synthetases, and dehydrogenases (Thompson et al., 1975; Stellwagen, 1976; Ryan & Vestling, 1974; Durgett & Greenley, 1977; Chambers & Dunlap, 1979; Böhme et al., 1977; Edwards & Woody, 1979). The ability of nucleotides to quench the dye vs. dye + enzyme difference spectra has been taken as evidence that Cibacron Blue binds to the nucleotide binding sites on the enzymes (Stellwagen, 1977). If the dissociation constant of the ADP–protein complex is known, one may calculate the dissociation constant of the dye–GS complex from measurements of the amplitude of the dye vs. dye + GS difference spectrum as a function of ADP concentration according to the following equation which was derived by C. Y. Huang:

$$K_{ADP} = \frac{yK_D[D][I]}{K_D([D_I] - y[D]) + [D][D_I](1 - y)}$$

where K_{ADP} = dissociation constant of the ADP–GS complex (=3 × 10^{-6} M), [D] = free dye concentration in absence of ADP, $[D_I]$ = free dye concentration in presence of ADP, [I] = total concentration of ADP, y = fractional change in dye bound to GS, and K_D = dissociation constant of the dye–GS complex. The calculated constants for the GS complexes of all four dye fractions were of the order of 10^{-7} M. Such calculated values are only approximations because of the following: (a) With all four dye fractions, a maximum of only 85% of the observed difference spectrum could be quenched by large excesses (20-fold) of ADP. Therefore, up to 15% of the spectral perturbation is due to dye which either is irreversibly bound to the ADP binding site or is bound to sites other than the ADP binding site. (b) An undetermined part of the spectral difference is caused by shifts in the dye–dye stacking equilibrium (Federici et al., 1985). (c) Results of calorimetric measurements show that there is a significant interaction between ADP and unfractionated Cibacron dye (ΔH = −0.7 to −0.8 kcal/mol), which should influence the free dye concentration (E. G. Gorman, personal communication). These considerations notwithstanding, the K_d value of 10^{-7} M which was calculated from the ADP competition studies is of the same order of magnitude as that for unfractionated dye preparations (K_d = 0.55 μM) as determined by direct binding measurements, and a K_i value of 0.85 μM as determined from kinetic measurements of the ability of unfractionated Cibacron Blue to inhibit the γ-glutamyltransferase activity of GS (Federici et al., 1985).

Irreversible Inactivation of GS by Fraction I. As was noted above, fraction I differs from the other three dye fractions in that immediately after it is bound to GS there is a slow change in the dye vs. dye + GS difference spectrum (Figure 6) which reflects covalent binding of the dye to the enzyme. The covalent binding of dye to GS is accompanied by a loss in the ability of ADP to displace dye from the enzyme. As shown in Table I, prolonged incubation (18 h) with fraction I at 25 °C led to 80% loss of the catalytic activity of both highly adenylylated ($GS_{\bar{11}}$) and relatively unadenylylated ($GS_{\bar{1}}$) forms of the enzyme. Negligible losses (≈2%) occurred in parallel incubation of GS in the absence of dye. Inclusion of 1.2 mM ADP–Mn in the incubation mixtures offered partial protection against the dye-induced inactivation. The somewhat greater protection of $GS_{\overline{2.0}}$ by ADP compared to that of $GS_{\bar{11}}$ probably reflects the fact that $GS_{\overline{2.0}}$ has a higher affinity for ADP than does $GS_{\bar{11}}$ (Hunt et al., 1975). By comparison, incubation of $GS_{\overline{2.0}}$ with fraction III led to only 10% loss of

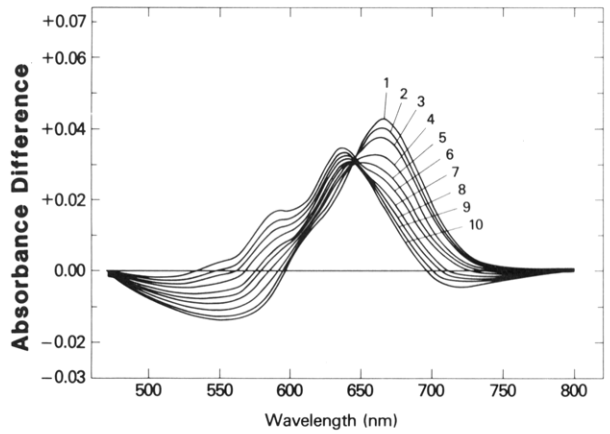

FIGURE 6: Time-dependent change in the difference spectrum between fraction I and fraction I + GS. The base line (zero change in absorbance) was obtained for the dye reaction mixture [14.0 μM fraction I, 100 mM KCl, 1 mM $MnCl_2$, and 35 mM Hepes (pH 7.5), ~25 °C] in both the sample and reference cuvettes by using the automatic base-line function of the instrument. The difference spectrum is produced for the native enzyme by the addition of 0.10 mL of 15–20 mg/mL stock protein solution (GS_I) to the sample and final dialysate to the reference, respectively. The spectrum was scanned with time (in minutes) after enzyme addition as follows: 1 = 2 min; 2 = 15 min; 3 = 30 min; 4 = 60 min; 5 = 90 min; 6 = 120 min; 7 = 180 min; 8 = 240 min; 9 = 300 min; 10 = 380 min.

Table I: Relationship between the Transferase Activity of GS and the Irreversible Binding of Fractions I and III in the Presence and Absence of ADP[a]

incubation mixture	transferase activity (units)	dye-promoted inactivation (%)	dye bound/GS subunits (mol/mol)
$GS_{\overline{11}}$	27.6		
+fraction I	5.8	79	0.92
+fraction I + ADP	14.3	54	
$GS_{\overline{2.0}}$	34.0		
+fraction I	7.7	78	0.86
+fraction I + ADP	25.1	28	
+fraction III	30.5	10	0.5

[a] Samples of GS (0.15 mL of $GS_{\overline{11}}$ and 0.14 mL of $GS_{\overline{2}}$) were added to the dye fraction I or III reaction mixture [100 μM dye, 35 mM Hepes (pH 7.5), 1 mM $MnCl_2$, and 100 mM KCl] to yield a final protein concentration of ~1.7 mg/mL in a volume of 1.15 mL. Identical incubations were performed in the reaction mixture described above plus the addition of 1.21 mM ADP–Mn. Control samples were incubated in the reaction mixture described minus dye. Aliquots of the incubation mixture (200 μL) were removed after incubation of ~18 h, 25 °C, applied to Sephadex G-50 columns (0.7 × 4.0 cm), and eluted with buffer [35 mM Hepes (pH 7.5), 100 mM KCl, and 1 mM $MnCl_2$]. Fractions (~0.5 mL) were collected and assayed for GS activity. Control values (minus dye) reflect the amount of GS activity applied to the columns and are uncorrected for the negligible loss of activity during chromatography (<2%). Inactivation values represent the mean ± the standard error for duplicate determinations.

activity. To determine the stoichiometry of dye binding, each of the dye-containing incubation mixtures described in Table I was dialyzed for 2 weeks against 35 mM Hepes (pH 7.5) and 100 mM KCl (400 volumes with five changes of buffer). For samples incubated in the absence of ADP, the ratio of bound dye to GS monomeric subunits was 0.9 and 0.86 for $GS_{\overline{11}}$ and $GS_{\overline{2.0}}$, respectively.

It is noteworthy that although the prolonged incubation of fraction III with $GS_{\overline{2.0}}$ led to only about 10% inactivation of the enzyme, about 0.5 equiv of the dye was irreversibly bound to the enzyme protein. This likely reflects a very slow nonspecific interaction of the dye to a site(s) other than the catalytic site.

Discussion

Weber et al. (1979) demonstrated that commercial preparations of Cibacron Blue F_3GA are heterogeneous mixtures of dye species. By means of column chromatography, they obtained apparently homogeneous preparations of one minor and one major component. Probably their minor component corresponds to our fraction I, and their major component corresponds to our fraction III or IV, or possibly to a mixture of these two fractions. In the thin-layer chromatographic system which they developed, the R_f's of their minor and major components were reported to be 0.77 and 0.57, respectively. In the same system, the R_f's of our fractions I, III, and IV were 0.83, 0.69, and 0.65, respectively. As discussed below, the discrepancy between the two sets of data may reflect differences in the purity of the comparable fractions (see below). This notwithstanding, it appears significant that the minor component of Weber et al. reacts irreversibly with phosphoglycerate kinase and isoleucyl-tRNA synthetase to inactivate these enzymes (Weber et al., 1979). Similarly as shown here, fraction I reacts irreversibly with GS to inactivate the enzyme. Such irreversible inactivations were not seen with the major component of Weber et al. or with our fractions II, III, or IV.

The covalent attachment of fraction I to GS involves at least two steps. First, the dye forms a reversible complex with the enzyme. This complex formation is associated with the rapid appearance of a characteristic peak at 665 nm in the dye vs. dye + GS difference spectrum. The ability of ADP to quench the difference spectrum immediately after mixing dye with the enzyme attests to the reversibility of the primary complex and also to the likelihood that the dye is bound to the nucleotide binding site on the enzyme. The primary complex formation is followed by a slow secondary reaction which can be monitored by a progressive shift in the difference spectrum and a loss in the ability of ADP to displace the dye from the enzyme. Since only 1 equiv of dye is ultimately bound per GS subunit, it seems likely that the dye becomes covalently bound at or near the primary (nucleotide) binding site. It thus appears that fraction I serves as an active-site reagent and may prove useful in structural studies on the catalytic site of the enzyme.

As already noted, fraction II is the fraction of choice to monitor the interconversion of the taut and relaxed forms of GS and may be used to discriminate between native and oxidized forms of GS. Differences in the specific activities of apparently homogeneous preparations of GS isolated from different batches of *Escherichia coli* probably reflect the presence of variable amounts of the oxidized (inactive) forms of GS (Levine, 1983). It appears that this inactive species is produced in vivo by the action of one or more mixed-function oxidase systems that catalyze the oxidation of a single histidine residue and another as yet unidentified amino acid residue in each subunit of the enzyme. This inactivation, which is characteristic of other enzymes as well (viz., kinases, dehydrogenases, and synthetases), may represent a regulatory step in the proteolytic degradation (turnover) of these enzymes (Oliver et al., 1983; Fucci et al., 1983). It is therefore of special interest that fraction II may be useful in the detection and quantification of the oxidized form of GS, and possibly other enzymes as well.

After the present study was completed, it was demonstrated by HPLC that none of the apparently homogeneous dye fractions used in this study is pure. In addition to a major component, each fraction contained lesser amounts of several other dye species. This discovery does not negate the important

conclusion from this work. The study confirms that commercial preparations of Cibacron Blue are heterogeneous and demonstrates that by difference spectroscopy various subfractions differ in their abilities to discriminate between various conformational states of GS. It is evident that further studies along these lines should be deferred until dye preparations of high purity can be obtained.

Registry No. GS, 9023-70-5; ADP, 58-64-0; Cibacron Blue F_3GA, 12236-82-7.

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Glucose-6-phosphate Dehydrogenase from *Saccharomyces cerevisiae*: Characterization of a Reactive Lysine Residue Labeled with Acetylsalicylic Acid†

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Received July 11, 1984

ABSTRACT: Glucose-6-phosphate dehydrogenase from *Saccharomyces cerevisiae* (bakers' yeast) reacts with acetylsalicylic acid, and this is accompanied by inactivation and modification of essentially one lysine residue per subunit. The amino acid sequence of an 11-residue tryptic peptide containing the reactive lysine residue of the yeast enzyme is given and establishes the existence of different subgroups of glucose-6-phosphate dehydrogenases. Thus, the labeled yeast structure has few similarities to the known structure around the reactive lysine residue of the enzyme from *Leuconostoc mesenteroides*, although it has extensive similarities with a structure in the human enzyme. It is further shown that amino acid sequences around reactive lysine residues of dehydrogenases in general vary, even though similarities occur around reactive lysine residues in 6-phosphogluconate, glutamate, and glyceraldehyde-3-phosphate dehydrogenases.

The importance of glucose-6-phosphate dehydrogenase as an enzyme of the pentose phosphate pathway has been recognized for almost 50 years [cf. Horecker (1976)], but relatively few structural studies have been reported [cf. Levy (1979)]. For the "classical" enzyme from human erythrocytes, a tentative, 495-residue primary structure showing most of the subunit amino acid sequence has been published (Beutler, 1983) without experimental evidence. The sequence determined for a C-terminal heptapeptide (Descalzi-Cancedda et al., 1984) does not agree with this sequence regarding the actual C-terminal structure (Lys-Leu replacing Gly) and possibly another position (a tryptic cleavage site replacing Leu).

The enzyme from the prokaryotic organism *Leuconostoc mesenteroides* has been labeled with pyridoxal 5′-phosphate, and an eight-residue sequence including a labeled lysine residue has been established (Haghighi et al., 1982). The enzyme from this source has also been crystallized in a form suitable for X-ray crystallographic determination of the three-dimensional structure (Adams et al., 1983). The *Leuconostoc* enzyme is one of the glucose-6-phosphate dehydrogenases that utilizes either NAD^+ or $NADP^+$, in contrast to the enzymes from eukaryotic and most prokaryotic cells, which are $NADP^+$ specific or $NADP^+$ preferring (Levy, 1979). It is not known whether this difference in functional behavior occurs despite clearly related primary structures, as in the case of the

† This work was supported by grants from the Wellcome Trust, the Swedish Medical Research Council (Project 13X-3532), the Karolinska Institutet, and the Knut and Alice Wallenberg Foundation.

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0006-2960/85/0424-0666$01.50/0 © 1985 American Chemical Society