Spectroscopic and Kinetic Analysis of a Monoclonal IgG Cryoglobulin. Effect of Mild Reduction on Cryoprecipitation†

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ABSTRACT: The precipitation of a monoclonal IgG2 crystalline cryoglobulin (WEB) is shown to be highly dependent on temperature and concentration. Below a critical concentration of 0.6 mg/mL there is no cryoprecipitation. The kinetics of the aggregation exhibits a concentration-dependent lag time. This evidence suggests that a nucleation event is important in the precipitation. Circular dichroism (CD) was used to investigate the conformational properties of the protein. At a low concentration (0.12 or 0.15 mg/mL), no detectable spectral changes in the far- and near-UV range were noted between 40 and 3 °C. However, at higher concentrations (1.21 mg/mL), a small and rapid CD change was observed in the 250–280-nm region at 3 °C. This indicates an intermolecular interaction that precedes the precipitation. Cryoprecipitation of WEB was also shown to be dependent on maintenance of intact interchain disulfide bonds. Only one or two interchain disulfides need be cleaved to abolish cryocrystallization and to significantly diminish the CD change at 3 °C. The evidence is consistent with the formation of an initial intermediate that involves interactions near the disulfide bonds in the hinge region of the cryoimmunoglobulin. In this model, cleavage of these disulfides prevents this interaction and abolishes cryoprecipitation.

Immunoglobulins which demonstrate reversible insolubility upon cooling are called cryoglobulins (Lerner & Watson, 1947). These proteins are clinically important because of their association with various immunological and lymphoproliferative disorders (Meltzer & Franklin, 1966; Grey et al., 1968; Brouet et al., 1974; Bengtsson et al., 1975) and the potential hazard they present due to their presumed ability to form immune complexes or aggregates in vivo. Investigation of cryoglobulins has shown that the cryoprecipitation phenomenon is dependent upon numerous factors which include the pH, temperature, and ionic strength of the solvent, the cryoprotein concentration in solution, and the time at which the cryoprotein solution remains at a given temperature (Saha et al., 1968; Pruzanski et al., 1973).

Recent investigations have clarified and defined particular solute and solvent effects which are related to the cryoprecipitation phenomenon (Middaugh & Litman, 1977a,b). In addition, there have been three studies of cryoprecipitable proteins that demonstrate that a low temperature induced conformational change may be a necessary step in the polymerization reaction (Middaugh et al., 1977; Saluk & Clem, 1975; Klein et al., 1977). However, recent evidence indicates that the majority of cryoimmunoglobulins do not undergo conformational changes at low temperatures (Middaugh et al., 1978; Scoville & Turner, 1978).

For a better understanding of the cryoprecipitation phenomenon, a monoclonal IgG2 cryocrystal protein (WEB) has been investigated. The clinical course and history of this patient and preliminary amino terminal amino acid sequences of the light and heavy chains have been previously reported (Johnston et al., 1975; Podell & Abraham, 1978). This paper describes the conformational properties of WEB as investigated by circular dichroism (CD)[1] and the kinetics of precipitation as followed by turbidity measurements. In addition, the effect of sequential reduction of WEB interchain disulfides and its relationship to its cryocrystalline properties were also studied.

Materials and Methods

Materials. Dithiothreitol (DTT) was obtained from Sigma. α-Iodoacetamide (IAA) and [1-^{14}C]-α-iodoacetamide were purchased from Aldrich and New England Nuclear, respectively.

Isolation and Purification of Immunoglobulins. IgG2-WEB was precipitated from plasma after incubation for 24 h at 4 °C and isolated by centrifugation at 12100*g* for 20 min at 4 °C. The partially purified cryocrystalline precipitate was dispersed and washed in 0.1 M borate buffered 0.15 M NaCl and 0.1% azide, pH 7.8 (BBS). The entire procedure was repeated 2–3 times. Then, in order to remove any trace of contaminating serum proteins, we solubilized the cryoprecipitate at 37 °C in BBS, dialyzed it against 0.015 M phosphate buffer, pH 7.4, and purified it by DEAE-cellulose chromatography at 37 °C in the same buffer. The IgG-WEB was obtained in the column fall through, dialyzed against BBS, and stored at 4 °C. Purity was checked by $NaDodSO_4$–polyacrylamide gel electrophoresis by using 0.1% $NaDodSO_4$ and 7.5% acrylamide (Steiner & Blumberg, 1971).

Protein Concentrations. Protein concentrations were determined spectrophotometrically at 280 nm by using the following extinction coefficients ($E_{1cm}^{1\%}$): WEB IgG (BBS without azide) = 16.5, WEB heavy chain (1 M acetic acid) = 15.4, and WEB light chain (1 M acetic acid) = 9.88. These were measured by using a micro-Kjeldahl analysis for total nitrogen content and by assuming the protein was 16.4% nitrogen (Kabat & Mayer, 1961). This latter value was

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[1] Abbreviations used: CD, circular dichroism; DTT, dithiothreitol; IAA, iodoacetamide; H, immunoglobulin heavy chain; L, immunoglobulin light chain.

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derived from the sequence of IgG-Eu (Edelman et al., 1969). It is also consistent with amino acid analysis of WEB heavy chain (unpublished experiments). Samples prepared for CD were filtered (Millipore SC filter; 8-μm pore size) before reading the absorbances.

Cryoprecipitation Assays. To determine the effect of concentration on cryoprecipitation, samples were incubated for 15 days at 4 °C. Immediately after this incubation period, the samples were centrifuged at 27000*g* for 30 min at 4 °C. The supernatant was aspirated and set aside for analysis. A volume of BBS equal to the original sample volume was then added to each of the pellets. These solutions were incubated at 37 °C for 24 h prior to analysis. The total protein concentration in the supernatant and the precipitate was determined by using a Lowry assay (Lowry et al., 1951).

To measure the effect of temperature on cryoprecipitation, 2.1 mg/mL samples of WEB buffered in BBS were incubated for 18 days at different temperatures in constant temperature rooms (±0.5 °C). These samples were then centrifuged at 27000*g* for 30 min at their respective temperatures. The supernatant was aspirated and its optical density determined. The formula for calculating percent cryoprecipitation has been previously reported (Middaugh & Litman, 1977b).

Turbidity Measurements. Turbidity at 350 nm due to formation of the cryocrystalline precipitate was measured with a Gilford 250 spectrophotometer interfaced to a PDP 11/34 computer. After incubation at 37 °C for at least 24 h, the 1-mL samples were added to the cuvettes and immediately placed in a prechilled cell chamber maintained at 3 °C. The temperature was simultaneously monitored by means of a thermistor in a cuvette containing only buffer. The temperature stabilized at 3 °C approximately 15 min after the samples were added.

Mild Reduction and Alkylation. Partial reduction of IgG-WEB was carried out in 0.2 M Tris-HCl buffer, pH 8.6, at protein concentrations of 2.9 or 4.3 mg/mL. DTT, dissolved in the same buffer, was added to aliquots of the protein in order to obtain molar ratios of DTT to protein which ranged from 0 to 68. All samples were adjusted to the same volume (which represented a 1–2% increase in the volume), incubated for 1 h at 37 °C, and divided into two portions. IAA, dissolved in Tris-HCl buffer, was added to one portion at a 20 molar excess to DTT. Recrystallized [1-^{14}C]-α-iodoacetamide (12.6 μCi/mmol) in Tris-HCl buffer was added to the other portion such that the molar ratios of radioactive IAA to DTT ranged from 10 to 20. All samples were adjusted to an identical volume. Control samples contained a 120-fold molar excess of [^{14}C]IAA or IAA to protein and no reducing agent. All samples were incubated for 90 min at 37 °C after the addition of the alkylating agent. Excess iodoacetamide was removed by extensive dialysis of the solutions at 37 °C against BBS in preparation for CD spectroscopy.

Samples which contained [^{14}C]IAA were dialyzed at room temperature against 0.2M Tris-HCl buffered 6 M urea, pH 8.6. The radiolabeled samples were totally reduced and alkylated as follows. DTT in Tris–urea buffer was added to each sample at a 200-fold molar excess of DTT to protein, and the samples were incubated for 1 h at 37 °C. These were alkylated by addition of *nonradioactive* IAA dissolved in the same buffer (molar ratio of IAA/DTT = 5) and incubation for 90 min at 37 °C. These preparations were then dialyzed extensively against 1 M acetic acid and 3 M urea and applied to a Sephadex G-100 column equilibrated with the same solution, and the appropriate column fractions were assayed for radioactivity due to [^{14}C]IAA. Molar ratios of ^{14}C to heavy and light chains were determined from the equation

$$\frac{\text{mmol of [}^{14}\text{C]IAA}}{\text{mmol of protein}} = \frac{\text{dpm/mL sample}}{\text{dpm/mmol [}^{14}\text{C]IAA}} \; \frac{1}{\text{mmol of protein/mL}}$$

Circular Dichroism. Circular dichroism spectra were obtained with a Jasco J-40 spectropolarimeter interfaced to a PDP 11/34 computer. The temperature of the samples was regulated and maintained by use of water-jacketed cuvettes after allowing at least a 20-min equilibration time once the specified temperature was reached. The exact cell path lengths were determined by using standard potassium chromate solutions (Haupt, 1952). Each scan was performed 3 or 4 times and the results were averaged. No smoothing of the spectral curves was necessary. The data were base-line corrected and converted into mean residue ellipticity by using the equation

$$[\theta] = \frac{\theta M}{cl}$$

where θ is the observed ellipticity, M is the mean residue molecular weight (110), c is the protein concentration in grams per 100 milliliters, and l is the cell path length in decimeters.

Temperature Drop. Protein samples incubated overnight at 37 °C were placed in a water-jacketed CD cell kept at room temperature. After the temperature of the sample stabilized at 21 °C, the temperature was rapidly dropped to 3 °C by starting circulation from the temperature-controlled bath. Simultaneously, the CD of the sample was monitored at 269 nm.

Results

The marked effect of temperature and concentration on the solubility of WEB is shown in Figure 1. Below a critical concentration of 0.6 mg/mL, no precipitate is observed. The time course of this aggregation as measured by the turbidity of the solution is shown in Figure 2. The decrease in turbidity at long times is due to sedimentation of protein aggregates. The kinetics of the association exhibits a lag period that is strongly dependent on the concentration of IgG-WEB. For example, concentrations of 1.21 and 1.69 mg/mL result in lag times of 160 and 15 min, respectively.

In order to investigate the possibility that cryoprecipitation is triggered by a conformational change when solutions of WEB are cooled, we measured its CD spectrum as a function of temperature from 205 to 330 nm. At a concentration of 0.121 mg/mL, no significant spectral change was observed between 40 and 3 °C. Thus, there is no detectable intramolecular conformational change induced by temperature. However, when similar experiments were performed at a concentration of 1.21 mg/mL, a small but reproducible CD change was observed between 20 and 3 °C. This CD change was restricted to the 260–280-nm region, as shown in Figure 3. When the temperature of the sample was dropped rapidly from 20 to 3 °C, the spectral change exhibited a relaxation time ($1/e$) of less than 3 min, and no further changes were observed for at least 120 min. This observation, coupled with the time dependence of the turbidity described above, eliminates light scattering as a possible origin for this CD change. The fact that it is confined to a localized wavelength region also argues against scattering.

In order to determine if the results noted for WEB are unique for cryoglobulins, we obtained the CD spectra of 2 noncryoglobulins, IgG2-Pg and IgG3-Ho. At low protein concentrations (0.30 mg/mL) they did not show any spectral

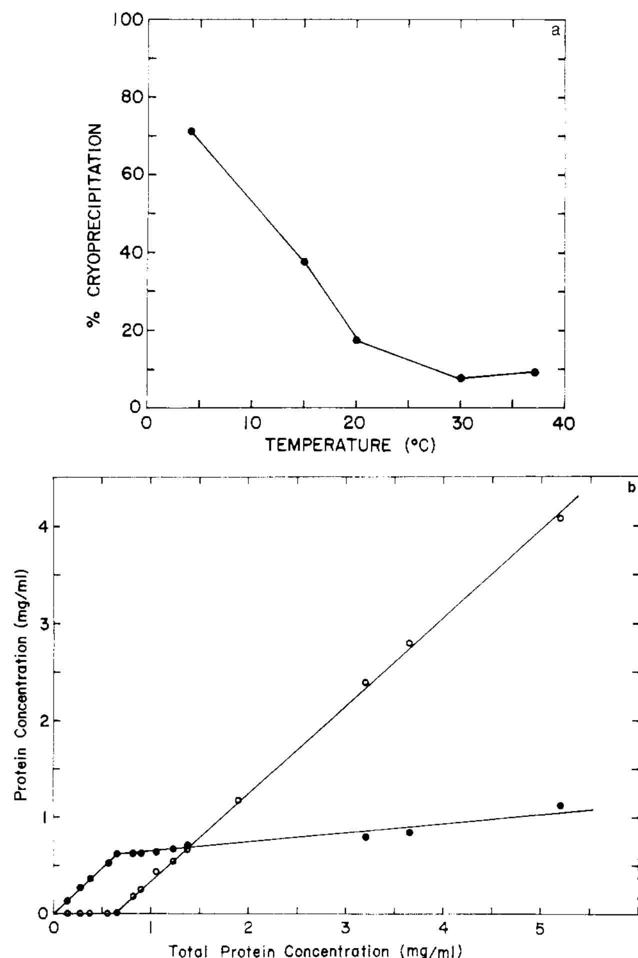

FIGURE 1: (a) Effect of temperature on the cryoprecipitation of WEB. All samples were at 2.12 mg/mL and incubated for 18 days. (b) Effect of concentration on the cryoprecipitation of WEB. All samples were incubated for 15 days at 4 °C. Symbols: (●) supernatant concentration; (O) precipitate concentration.

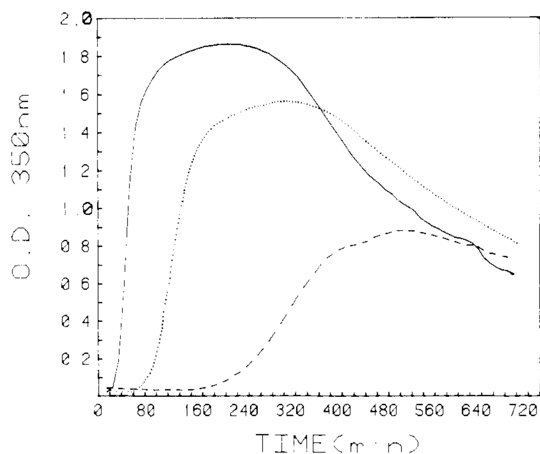

FIGURE 2: Turbidity at 350 nm as a function of time. Temperature is 3 °C. Concentrations of cryoglobulin WEB: (---) 1.21; (···) 1.45; (—) 1.69 mg/mL.

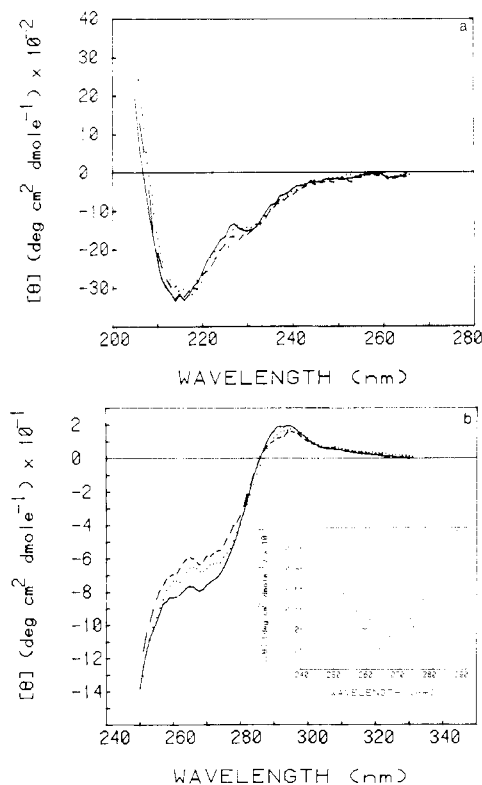

FIGURE 3: Circular dichroism spectra of cryoglobulin WEB. (a) Far-UV CD spectra, protein concentration 1.21 mg/mL, in 0.0065-cm cell path length. (b) Near-UV CD spectra, concentration 1.37 mg/mL, in 0.51-cm cell path length. Symbols: (---) 40; (···) 20; (—) 3 °C. Insert: Near-UV CD difference spectra of WEB at 1.21 mg/mL (3 °C minus 20 °C.)

change with temperature. At increased concentrations (1.21 mg/mL) a consistent and reproducible CD difference between the 3 and 20 °C spectra was observed. In the 260–280-nm region, the average magnitudes of the differences for IgG2-Pg and IgG3-Ho were about $^2/_3$ of that observed for WEB. Thus, the spectral change is not unique to the cryoglobulin.

In order to determine if the cryoprecipitability and/or the CD changes noted were related to the maintenance of interchain covalent disulfide bond structure in WEB, we studied the effects of mild reduction on the cryoprecipitability. For this group of experiments, the cryoglobulin at 2.1 mg/mL was reduced with DTT at DTT/protein molar ratios of 10:1, 17:1, 28:1, 40:1, and 68:1. These samples were then flushed with nitrogen for 30 min, stoppered, and incubated for at least 48 h at 4 °C. Only the control sample (no DTT added) cryoprecipitated. If the samples were allowed to reoxidize by standing in air at 4 °C, all the samples eventually cryoprecipitated. If they were alkylated with IAA after reduction and exposed to air at 4 °C, only the control sample cryoprecipitated.

The effect of mild reduction and alkylation on the CD spectrum of WEB is shown in Figure 4a,b. Only small changes are observed in the near-UV range (Figure 4a). At a 10:1 molar ratio, they are similar to the changes seen upon the loss of one or more disulfide chromophores in plasmin-modified human somatotropin (Bewley, 1977). There is also approximately a 10% change in the ellipticity of the 217-nm peak.

In order to determine if cleavage of the interchain disulfide bonds produced a change in the effect of temperature on the CD spectra, samples were studied after being reduced with gradually increasing concentrations of DTT and alkylated with IAA. CD spectra were obtained at 40, 20, and 3 °C for samples reduced with 0:1, 10:1, 17:1 and 68:1 molar excesses of DTT to protein. The difference spectra (3 °C minus 20 °C) of these samples are shown in Figure 5a. The CD difference spectra for the 10:1, 17:1, and 68:1 samples are similar and have reduced magnitudes relative to the control. This effect is seen more markedly when the difference ellipticity is averaged from 280 to 260 nm as shown in Figure 5b. (For these spectra over this small wavelength interval, this average is proportional to the difference in rotational strength

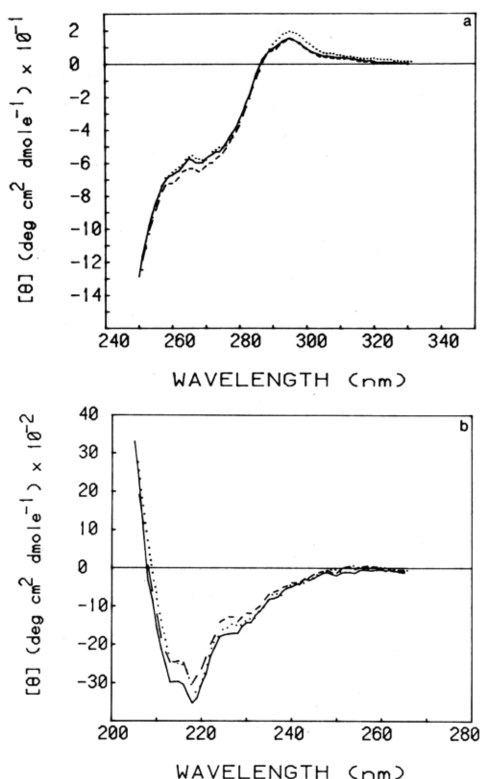

FIGURE 4: Circular dichroism spectra of unreduced and reduced/alkylated cryoglobulin WEB. Temperature is 40 °C. (a) Near-UV CD spectra, concentration 1.21 mg/mL, in 0.51-cm cell path length. (b) Far-UV CD spectra, concentration 1.21 mg/mL, in 0.0065-cm cell path length. Symbols: (---) unreduced WEB; (—) reduced WEB at a molar ratio of DTT/protein of 10:1; (···) reduced WEB at 68:1.

Table I: Quantitative Analysis of the Extent of Reduction of WEB

molar ratios of DTT to protein used for reduction	molar ratios of [1-^{14}C]IAA to heavy chain[a]	
	trial 1	trial 2
10:1	1.85	1.40
17:1	1.75	1.99
28:1	3.91	3.38
40:1	not done	3.76
68:1	4.3	4.2

[a] Molar ratios of [1-^{14}C]IAA to light chain at 10:1 and 28:1 were respectively 0.17 and 0.44 (trial 1) and 0.28 and 0.42 (trial 2).

to within about 2%.). The precipitous decrease in magnitude of this difference ellipticity correlated with the absence of cryoprecipitation in these samples when stored at 4 °C for a prolonged period.

The overall extent of reduction and alkylation occurring at various DTT levels can be seen by $NaDodSO_4$ slab gel electrophoresis under denaturing conditions as shown in Figure 6 (Steiner and Blumberg, 1971). The control samples primarily contain intact molecules which consist of two heavy and two light polypeptide chains (H_2L_2), with contamination by small amounts of H_2L, H_2, and trace amounts of L and H chains. The appearance of HL is seen upon reduction at 10:1. The 10:1 and 17:1 samples show that the inter-heavy-chain disulfide bridges were more labile to reduction than the inter heavy–light chain disulfides. This can be seen by the lack of any prominent H_2 band. A quantitative analysis of the extent of reduction was obtained by radioactively alkylating with [^{14}C]IAA (see Table I). At a DTT to protein ratio of 68, the average number of disulfides reduced was

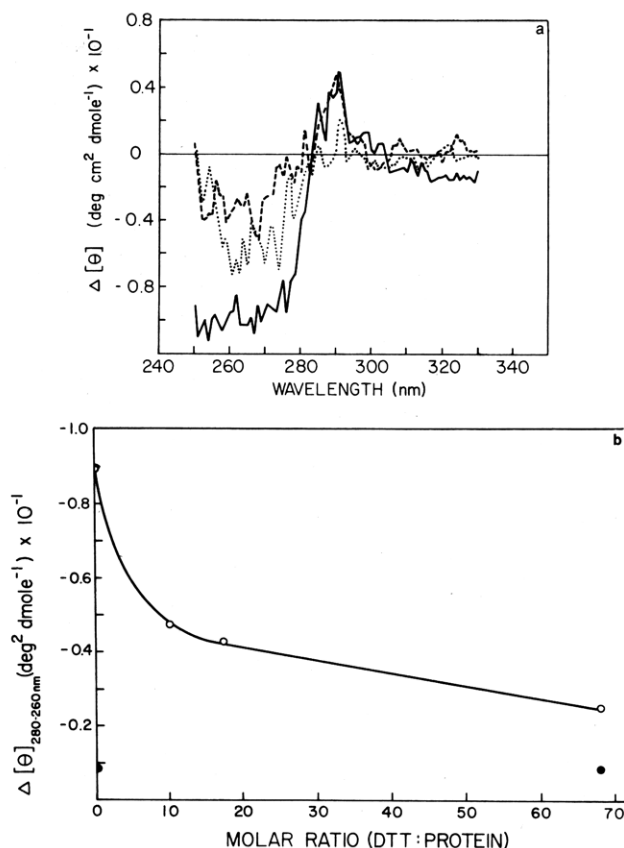

FIGURE 5: (a) Difference circular dichroism spectra of WEB (3 °C minus 20 °C) under varying molar ratios of DTT/protein. Symbols: (—) 0:1; (···) 10:1; (---) 68:1. (b) Average of CD difference spectra over the range 280–260 nm. Symbols: IgG2-WEB (O) 1.21 mg/mL; (●) 0.15 mg/mL.

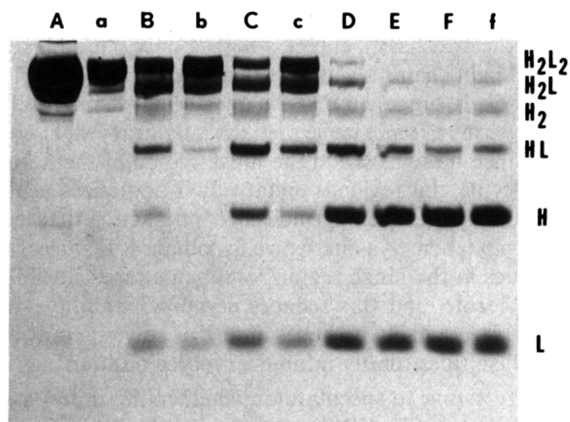

FIGURE 6: Slab gel electrophoresis of sequentially reduced and alkylated WEB. Samples, 1.21 mg/mL, were denaturated overnight by using 2% sodium dodecyl sulfate without any reducing agent. Gels are 7.5% acrylamide and 0.1% $NaDodSO_4$. No stacking gel was used. The entire protocol as described in the text was done twice. Symbols of the molar ratios of DTT protein: (A) 0:1; (B) 10:1; (C) 17:1; (D) 28:1; (E) 40:1; (F) 68:1. Lower-case letters represent the corresponding samples of the second trial.

approximately four per heavy chain. At a ratio of 10:1 where cryoprecipitation was abolished, an average of only 1.6 disulfides were reduced per heavy chain.

Discussion

The cryoprecipitation of WEB is extremely temperature dependent, as shown in Figure 1. However, the CD experiments at low concentrations indicate this is not due to a temperature-induced conformational change. Instead, it

appears that a cooperative intermolecular interaction is responsible. Two intermolecular associations have been demonstrated in this work. The first is the precipitation which is marked by a highly concentration dependent lag time and a critical concentration below which the protein is completely soluble (see Figures 1 and 2). These features are characteristic of protein associations that require a nucleation event (Williams, 1973; Moffatt & Gibson, 1974; Gaskin et al., 1974; Oosawa & Asakura, 1975; Johnson & Borisy, 1977). The second association is detected by CD and occurs before the precipitation. This type of association is also present in noncryoglobulins and in reduced WEB.

The cryoprecipitation is clearly dependent upon intact interchain disulfide bonds. Addition of DTT and storage under nitrogen or subsequent reaction with IAA completely abolished the cryoprecipitability. However, CD spectra obtained before and after alkylation indicated that only a small conformational change was induced by the reduction process (see Figure 4), which suggests that a localized region of the molecule is involved in the cryoprecipitation. The observation that reoxidized WEB does cryoprecipitate is consistent with this notion since it implies that the cysteines remain close enough to re-form the original bridges. Moreover, only a limited number of disulfide bonds need to be reduced since cryoprecipitation was shown to be totally inhibited when an average of 1.6 disulfides was cleaved in the intact molecules. The gels in Figure 6 indicated that these are interchain disulfide bridges since, as the reduction process proceeded, bands which corresponded in molecular weight to heavy (H), light (L), and HL (1/2 molecule) appeared, demonstrating that the chains were no longer covalently linked. Of particular note is the observation that the 1/2 molecule, or HL, became predominant at low levels of reduction, whereas a band which corresponded to H_2, i.e., disulfide-linked heavy chains, was nearly absent. This was true even though four disulfides must be cleaved to separate the two heavy chains whereas only one holds the heavy and light chains together (Frangione et al., 1969). This finding strongly suggested that the inter-heavy-chain disulfide bridges were much more labile than the inter heavy–light chain bonds. Since these reside in the hinge region of the normal IgG2 molecule, this region is tentatively hypothesized as that which is involved in the intermolecular interaction that leads to cryoprecipitation. An alternative hypothesis is that cleavage of disulfides in the hinge region results in altered flexibility of the molecule, and this reduces cryoprecipitability. We consider this less likely because rupture of only one to two of the four disulfides totally inhibits cryoprecipitation.

It is interesting to speculate on whether the initial association, as detected by CD, is necessary for cryoprecipitation. The data available are consistent with this possibility since the initial intermediate detected by CD was not observed at temperatures where there was no cryoprecipitation. Further, the effect of reduction on the magnitude of the CD change in concentrated solutions of IgG-WEB at temperatures from 20 to 3 °C paralleled the effects of reduction on cryoprecipitation (see Figure 5). The localization of the CD change to the 250–280-nm region was also consistent with the importance of disulfide bonds as revealed by chemical modification. The difference spectrum (see Figure 3 insert) is very similar to that observed upon cleavage of disulfide bonds in plasmin-modified human somatotropin (Bewley, 1977), and there is considerable additional evidence in other systems that disulfide bridges contribute to the optical activity in this region (Beychok, 1965, 1966; Beychok & Breslow, 1968; Ludescher & Schwyzer; 1971; Casey & Martin, 1972; Yamashiro et al., 1975). However, it must be pointed out that other chromophores are also important in determining the optical activity from 250 to 280 nm. Thus, the evidence is consistent with, but does not conclusively demonstrate, the formation of a required initial intermediate. This type of detail can only be established with further experiments.

In summation, the data indicate that cryoprecipitation of IgG-WEB does not require a temperature-dependent intramolecular conformational change. Rather, the temperature sensitivity of the protein and its cryoprecipitability result from a cooperative intermolecular association that is dependent upon the maintenance of disulfide bond structure in the involved region (presumed to be the hinge region). Obviously, the localization of these structures will depend upon structural analyses of selectively reduced and alkylated proteins. This work is now proceeding.

Acknowledgments

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Purification and Properties of a Translation Inhibitor from Wheat Germ†

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ABSTRACT: A translation inhibitor from wheat germ has been purified more than 400-fold to apparent homogeneity. The inhibitor is a basic protein with a molecular weight of 30000. This protein effectively blocks protein synthesis in animal cell-free extracts but does not affect protein synthesis in intact cells. Inhibition occurs at a ribosome to inhibitor molar ratio of 100:1, indicating an enzymic mechanism of action. The wheat germ protein inhibits the translation of endogenous mRNA, exogenous mRNA, and poly(uridylic acid) at a step in polypeptide chain elongation and without breakdown of the polysomes. Neither the aminoacylation reaction nor mRNA degradation is affected by the inhibitor. An interesting feature of the inhibition reaction is that it requires, in addition to the wheat germ inhibitor, both ATP and tRNA. The function of these two compounds in the inhibition is presently unknown since neither the hydrolysis of the β,γ-pyrophosphate bond of ATP nor a modification of the tRNA can be demonstrated during the reaction.

Cell-free extracts from animal cells and plant embryos have become useful systems for carrying out the translation of isolated mRNAs and for studying possible mechanisms involved in the regulation of protein synthesis (Lodish, 1976; Busch et al., 1976). These extracts prepared from different eucaryotic cell types are generally regarded as being qualitatively similar with respect to the components of their protein-synthesizing machinery. However, certain components, such as initiation factors and isoaccepting tRNA species, do vary quantitatively, and perhaps qualitatively, between species. These variations could explain the observation that a given mRNA may be translated with unequal efficiency and fidelity in different cell-free systems. For example, EMC[1] viral RNA is translated more efficiently in an ascites cell-free system than in a wheat germ system, whereas the reverse is true for many animal cell mRNAs.

An interest in this difference between the wheat germ and ascites cell-free systems prompted us to do mixing experiments between the two systems. These experiments failed because very small amounts of components from the active wheat germ system completely inhibited protein synthesis in the ascites cell extracts. We found that wheat germ extract stopped the translation of ascites cell mRNA in the ascites cell system at a concentration less than 0.1% of that present during the translation of the same mRNA in the wheat germ system (Stewart et al., 1977). This suggested the presence in the wheat germ extract of an inhibitor with an enzymic mode of action and precise species or kingdom specificity. We felt that such an inhibitor could be useful as a reagent for studying the molecular mechanisms of protein synthesis, as a probe for differences in the translational machinery of eucaryotes, and as a means of identifying a reaction that might prove to be a general mechanism for regulating protein synthesis.

In an earlier report, we presented evidence indicating that the wheat germ inhibitor is a protein and that it acts at a step in polypeptide chain elongation (Stewart et all., 1977). Here, we describe the purification of the inhibitor to apparent homogeneity. Also, we present additional evidence concerning the requirements for inhibitor activity as well as the inhibitor's mechanism of action.

Experimental Procedures

Preparation of Extracts. Wheat germ was obtained fresh from a local mill and stored at 4 °C. A cell-free protein-synthesizing system was prepared from this material according to the procedure of Marcu & Dudock (1975) except that KCl was replaced with potassium acetate in the buffers. This extract was found to efficiently translate poly(A)-containing RNA from ascites cells, but to inhibit, at low concentrations,

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[1] Abbreviations used: EMC virus, encephalomyocarditis virus; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; poly(U), poly(uridylic acid); $NaDodSO_4$, sodium dodecyl sulfate; AMP-PCP, adenylyl (β,γ-methylene)diphosphonate.

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