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Role of Disulfide Interchange Enzyme in Immunoglobulin Synthesis†

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ABSTRACT: The role of disulfide interchange enzyme in protein biosynthesis was evaluated by studying the enzyme from mouse lymphoid tissue. The enzyme isolated from lymphoid cells was shown to have no tissue-specific characteristics. It was identical with the enzyme synthesized by mouse liver in its biochemical and immunological properties and its capacity to promote both disulfide bond formation and insulin degradation. In contrast to liver, the levels of enzyme in lymphoid tissues were found to vary with immunoglobulin secretory activity. Assays of lymphoid cells and their transformed counterparts showed that the enzyme contents of cells actively secreting immunoglobulin were 1–2 orders of magnitude higher than that of unstimulated B cells or non-immunoglobulin-producing T cells. The increase in enzyme levels paralleled the increase in immunoglobulin synthesis after antigen or mitogen stimulation and was independent of the class of immunoglobulin produced. This correlation indicated that the enzyme plays a critical role in the formation of intramonomer bonds common to all immunoglobulin molecules. Supporting data were obtained by assaying the ability of the enzyme to promote the polymerization of mouse pentamer IgM in vitro. The enzyme was found to catalyze the formation of the interchain bonds required for monomer IgM assembly but not the formation of the intermonomer bonds required for pentamer assembly. The sum of these results provides strong evidence that disulfide interchange enzyme functions in the in vivo synthesis of protein disulfide bonds.

The physiological function of disulfide interchange enzyme (thiol:protein disulfide oxidoreductase) remains to be established. The enzyme has been implicated in the formation of protein disulfide bonds on the basis of considerable indirect evidence: its wide tissue distribution, its localization in the microsomal fraction, and its ability to convert inactive, randomly reoxidized proteins to their native forms (De Lorenzo & Molea, 1967; Fuchs et al., 1967; Goldberger et al., 1963, 1964; Steiner et al., 1965). As yet, however, there is little direct evidence that the enzyme performs such a function in vivo. One problem has been the purity of the preparations used in the investigations. Most previous studies have been carried out with partially purified enzyme so that it was not possible to distinguish catalytic from nonspecific primer effects (Murkofsky & Lamm, 1979; Della Corte & Parkhouse, 1973; Teale & Benjamin, 1976; Wilde & Koshland, 1978). Moreover, preparations of disulfide interchange enzyme have been found to exhibit a second thiol oxidoreductase activity (EC 1.8.4.2), the reductive cleavage of insulin disulfide bonds (Ansorge et al., 1973; Varandani et al., 1975; Morin et al., 1978). Whether the two activities are mediated by a single enzyme of broad specificity or by distinct enzyme species with overlapping specificities is a question that has yet to be resolved (Varandani, 1978; Freedman, 1979; Hillson & Freedman, 1980).

Investigations of the role of disulfide interchange enzyme have also been hampered by the lack of definitive assays for physiological activity. In the past, studies have been generally confined to systems such as liver protein biosynthesis, which have proved too complex to give clear-cut data. Only recently have studies been initiated on tissues that are metabolically more restricted and synthesize a single major disulfide-linked protein. By analyzing collagen and bone synthesis in chick embryos, Brockway et al. (1980) have been able to correlate the levels of disulfide interchange enzyme with the synthesis of the disulfide-linked procollagen.

We have undertaken to obtain definitive evidence for the role of disulfide interchange enzyme by using the assembly of mouse pentamer IgM as the functional assay. To address the problem of enzyme heterogeneity, disulfide interchange enzyme was purified not only from the homologous IgM-secreting lymphoid tissue but also from mouse liver, and the two preparations were compared with respect to substrate specificity and to biochemical and immunological properties. A radioimmunoassay was developed to follow the intracellular levels of disulfide interchange enzyme as a function of the successive differentiative steps in Ig[1] synthesis. Finally, the enzyme preparations were tested for their ability to promote the repolymerization of mouse IgM in vitro.

This system was chosen because the in vitro assembly of immunoglobulin (Petersen & Dorrington, 1974; Sears et al., 1977) has been shown to be representative of the in vivo process. Within the cell, the intrachain and interchain S–S bonds of Ig heavy and light chains are formed extremely rapidly, even in some cases as the polypeptides are being synthesized on membrane-bound ribosomes (Bergman & Kuehl, 1979a,b). Shortly after the completion of the synthesis of the chains, the remaining disulfide bonds are formed and the disulfide-linked pairs, either H–L, or L–L and H–H, are then disulfide bonded to assemble the basic monomeric unit of H_2L_2.[1] In the case of IgM, the monomers are transported

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[1] Abbreviations used: H, heavy chain; L, light chain; Ig, immunoglobulin; $NaDodSO_4$, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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to the Golgi apparatus or the plasma membrane. At these sites, the IgM monomers and a third polypeptide, the J chain, are cross-linked by disulfide bonds in a regular stoichiometry of one J chain per five monomers (Chapuis & Koshland, 1974; Koshland, 1975), and the polymer product is rapidly secreted (Parkhouse & Askonas, 1969; Parkhouse, 1971). It has been found that two of the intracellular steps, the assembly of monomer and pentamer, can be mimicked in vitro by mildly reducing the secreted pentamer and then reassembling the polymer under appropriate experimental conditions (Della Corte & Parkhouse, 1973; Wilde & Koshland, 1978). Moreover, analyses of both the in vivo and in vitro process have suggested that Ig assembly involves the rearrangement of disulfide bonds. The strongest evidence was the finding by Della Corte & Parkhouse (1973) that disulfide interchange enzyme is required for the repolymerization of mouse IgM. Thus, we have used the assembly of pentamer IgM as a model system for evaluating the contribution of disulfide interchange enzyme to both interchain and intersubunit bond formation.

Materials and Methods

Lymphoid Tumor and Cell Lines. The plasmacytomas MOPC 104E ($\lambda\mu$), S194A ($\kappa\alpha$), S134 ($\kappa\gamma_{2b}$), P1 ($\kappa\gamma_{2a}$), Y5606 ($\lambda\gamma_3$), and P3 ($\kappa\gamma_1$) were kindly provided by Dr. Anne Good, University of California, Berkeley, CA, the plasmacytomas TEPC 183 ($\kappa\mu$) and HPC 76 ($\kappa\mu$) by Dr. L. Herzenberg, Stanford University Medical School, Stanford, CA, and the plasmacytoma MOPC 315 ($\lambda\alpha$) by Dr. E. Simms, Washington University Medical School, St. Louis, MO. The thymomas S49 and W7 were the gifts of Dr. R. Mishell, University of California, Berkeley, CA, and the B lymphoma WEHI 231 was the gift of Dr. Noel Warner, University of New Mexico, Albuquerque, NM. The plasmacytomas were grown subcutaneously in BALB/c mice and harvested prior to attaining 1 cm in diameter. The thymomas and the B lymphoma were grown in tissue culture media (Raschke et al., 1979).

Enzyme Purification. Disulfide interchange enzyme was purified from the livers of BALB/c mice (4-month-old females) and from HPC 76 plasmacytoma tumors grown in BALB/c mice to approximately 1 cm in diameter. The purification was carried out by the procedure developed by Carmichael et al. (1977) for bovine disulfide interchange enzyme. The procedure consisted of lysis of the cells and extraction of the enzyme by homogenization of the cells in a Waring blender in the presence of 1% Triton X-100. The homogenate was centrifuged at 28000*g* for 15 min, and the resulting supernatant was heated at 60 °C for 15 min. The heat-treated material was centrifuged for 15 min at 25000*g*, and the supernatant was further fractionated by ammonium sulfate precipitation. The material that precipitated between 60% and 85% saturated ammonium sulfate contained the enzyme activity and was further fractionated by chromatography on CM-Sephadex C-50 at pH 5.15, Sephadex G-200, and DEAE-Sephadex A-50 at pH 7.5.

Enzyme Assays. The enzyme preparations were assayed for the ability to degrade insulin by a modification of the method described by Carmichael et al. (1977). Insulin labeled by the chloramine T method with ^{125}I was diluted with unlabeled carrier insulin to a specific activity of 200 μCi/g. Aliquots containing 80 μg were brought to a volume of 0.5 mL in 0.1 M sodium phosphate buffer, pH 7.5, 5 mM ethylenediaminetetraacetic acid, 0.1% (w/v) bovine serum albumin, and 1 mM reduced glutathione. The solutions were heated to 37 °C, the enzyme was added, and after 5 min, the reaction was terminated by the addition of 0.25 mL of 15% (w/v) trichloroacetic acid. The precipitate formed after 1 h at 0 °C was removed by centrifugation, and aliquots of the supernatant were taken to measure the amount of acid-soluble radioactivity generated. Several dilutions of the samples were assayed to ascertain that the enzyme concentration used was within the linear portion of the curve. Control samples without enzyme were included to determine any nonenzymatic increase in acid-soluble radioactivity. Samples were counted in a Nuclear-Chicago γ counter, Model 1085 (efficiency 76%) to an accuracy of at least 2%. A unit of enzyme activity was defined as that amount which degrades 1 μg of insulin to trichloroacetic acid soluble fragments under the above conditions.

Polymerization Assay. Purified pentamer IgM (1.35 mg) was reduced with 3 mM dithioerythritol for 1 h at 22 °C, and the free reducing agent was removed by passage of the solution over a 1.5 × 5 cm Sephadex G-25 column equilibrated in 0.15 M NaCl, 20 mM Tris-HCl, pH 7.5, and 0.02% sodium azide. Fifteen-microliter aliquots containing 25 μg of the reduced IgM were then incubated for 1 h at 37 °C with 50 μL of the enzyme or cell extract sample to be tested. The reaction was terminated by alkylation with 50 mM iodoacetamide, and the extent of polymerization was determined by $NaDodSO_4$–agarose–acrylamide gel electrophoresis as described previously (Wilde & Koshland, 1978). One polymerization unit was defined as the amount of material capable of polymerizing 10 μg of IgM under the conditions described for the assay.

Radioimmunoassay for Disulfide Interchange Enzyme. Anti-disulfide interchange antibody was prepared by injecting New Zealand white rabbits subcutaneously with 100 μg of purified mouse liver enzyme emulsified in complete Freund's adjuvant. One month after the second injection, the rabbits were bled from the marginal ear vein, and the Ig fraction of the sera was isolated by ammonium sulfate precipitation. Samples of spleen, tumor, and liver cells were prepared by disrupting the tissue between two glass slides, lysing the red blood cells present with buffered ammonium chloride, and removing dead cells and cell debris by passage over glass wool columns (Mishell & Shiigi, 1980). The spleen cells were further fractionated by 1*g* velocity sedimentation, and the small and large cell fractions were taken for analysis (Miller & Phillips, 1969). Cell viabilities as determined by Trypan blue exclusion were over 80% except for the liver cells, which ranged from 20% to 40% viable. The cell suspensions were counted and centrifuged 15 min at 1000*g*, and the cell pellets were resuspended in 1% Nonidet P-40. After 1 h at 4 °C, the lysed cells were centrifuged 30 min at 50000*g*, and the supernatants were used in the radioimmunoassays.

Twofold dilutions of the cell lysates were mixed with an amount of rabbit anti-disulfide interchange enzyme antibody that was known to give 50% binding of 2 ng of the purified liver enzyme. After 1-h incubation at 37 °C, 2 ng of ^{125}I-labeled liver enzyme, radioiodinated by the lactoperoxidase method (Morrison & Bayse, 1970), was added to each tube, and the incubation was continued for 1 h at 37 °C and 16 h at 4 °C. The antigen–antibody complexes were then precipitated by facilitation with an equivalence amount of goat anti-rabbit globulin. The precipitates were collected by centrifugation and washed 1 time, and their ^{125}I content was determined in a Nuclear-Chicago γ counter to an accuracy of 1%. All dilutions were made in 80 mM sodium phosphate buffer, pH 7.2, containing 0.5% Nonidet P-40, 0.5% bovine serum albumin, and 0.02% sodium azide, and the reaction volume was kept constant at 0.9 mL. The amount of disulfide interchange enzyme in the cell lysates was calculated by comparing the volume that gave 50% inhibition of binding,

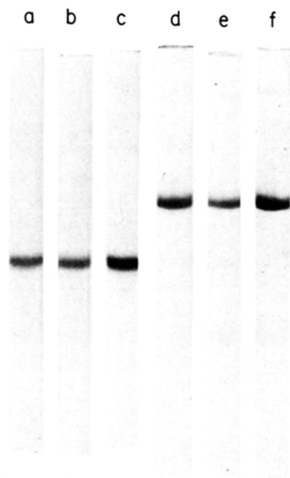

FIGURE 1: One-dimensional electrophoretic analysis of purified disulfide interchange enzyme. Ten micrograms of (a) liver enzyme, (b) plasmacytoma enzyme, or (c) a mixture of both was electrophoresed on 5% $NaDodSO_4$–polyacrylamide gels, and 10 μg of (d) liver enzyme, (e) plasmacytoma enzyme, or (f) a mixture of both was electrophoresed on 7.5% alkaline pH polyacrylamide gels.

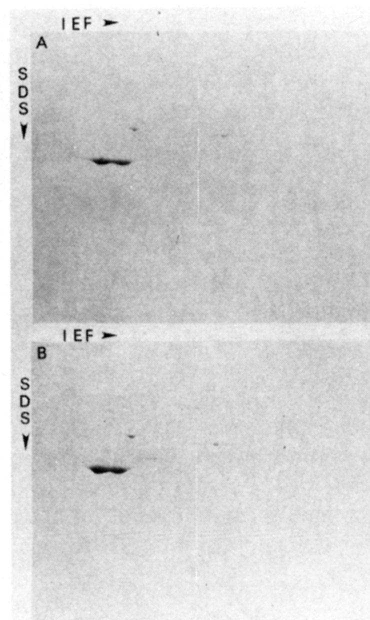

FIGURE 2: Two-dimensional electrophoretic analyses of purified disulfide interchange enzyme. Two micrograms of (A) liver enzyme or (B) plasmacytoma enzyme was electrophoresed on isoelectric focusing (IEF) gels first and then on 12.5% $NaDodSO_4$–polyacrylamide gels.

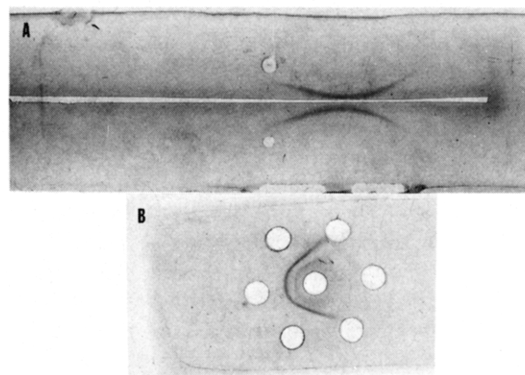

FIGURE 3: Immunological analyses of purified disulfide interchange enzyme. (A) Ten micrograms of liver enzyme (upper well) and 10 μg of plasmacytoma enzyme (lower well) were electrophoresed and then developed with antibodies to the liver preparation. The cathode is on the left-hand side. (B) Ten micrograms of liver enzyme was placed in each of the two wells flanking 10 μg of plasmacytoma enzyme. Antibodies to the liver enzyme were placed in the center well.

with the equivalent endpoint obtained by titrating known concentrations of purified liver enzyme.

Analytical Procedures. Amino acid analyses of the purified enzymes were performed on a Beckman Model 120C analyzer after hydrolysis for 20 h under vacuum in 6 N HCl (Koshland et al., 1966). The protein concentrations of the purified enzymes were determined from these analyses so that the enzyme solutions could serve as standards in the dye binding assay used to measure the protein concentration of cell lysates (Bradford, 1976). Alkaline gel electrophoresis was performed in a discontinuous gel system on 7.5% polyacrylamide gels at a running pH of 9.5 (Reisfeld & Small, 1966). For estimation of molecular weights, the enzyme preparations were boiled 1 min in 1.0% $NaDodSO_4$ and 1% 2-mercaptoethanol and then layered on 5.0% polyacrylamide gels in the presence of 1% $NaDodSO_4$ (Weber & Osborn, 1969). The mobility of the enzymes was compared with those of the marker proteins, μ chain, γ chain, ovalbumin, bovine serum albumin, DNAse, and RNAse. Two-dimensional gel electrophoresis was performed as detailed by O'Farrell (1975) where the first dimension was an isoelectric focused gel (pH 4–7) and the second gel was a 12.5% polyacrylamide–$NaDodSO_4$ gel.

Results

Characterization of Mouse Disulfide Interchange Enzyme. Disulfide interchange enzyme was isolated in good yield from mouse plasmacytomas secreting IgM and mouse liver. An average of 4 mg of purified material was obtained per 100 g of tumor tissue and one-half that amount, 2 mg, per 100 g of liver tissue. The preparations appeared homogeneous in disc gel electrophoresis; only a single band was observed when 10-μg samples were analyzed on $NaDodSO_4$ or alkaline urea–polyacrylamide gels (Figure 1). Small differences in charge were detected, however, by the more sensitive technique of two-dimensional electrophoresis (O'Farrell, 1975). As shown in Figure 2, the enzyme migrated as a doublet with an isoelectric point ranging from 4.0 to 4.5. This microheterogeneity could not be attributed to allelic differences because the enzymes were isolated from inbred strains of mice. It seemed likely, therefore, that the charge differences were caused by partial deamidination of the enzyme and/or by variations in its oligosaccharide content. In addition to the major enzyme component, a few minor contaminants were also detected by two-dimensional electrophoresis. From the staining intensities of the contaminants, it was estimated that the plasmacytoma enzyme was at least 90% pure and the liver enzyme more than 95%. These estimates of purity were supported by the results of immunological analyses. As illustrated in Figure 3, the enzymes preparations were examined by immunodiffusion and immunoelectrophoresis by using a rabbit antibody prepared against the mouse liver enzyme. The finding of a single precipitin arc in every case provided further evidence that the preparations were composed of a single major species of protein.

The purified disulfide interchange enzymes were found to be highly susceptible to proteolytic attack. Degradation was first observed in samples that were stored at 4 °C without the addition of proteolytic inhibitors. Electrophoretic analyses showed that during storage more than 90% of the enzyme was converted from its native form of 60 000 daltons to a fragment of approximately 40 000–45 000 daltons. Presumably the

Table I: Amino Acid Composition of Disulfide Interchange Enzyme[a]

amino acid	mouse liver DSI[b]	plasma-cytoma HPC 76 DSI[b]	beef liver DSI[b,c]
lysine	46	45	48
histidine	10	10	12
arginine	12	13	11
aspartic acid	54	54	55
threonine	23	22	21
serine	22	24	24
glutamic acid	68	71	66
proline	22	18	21
glycine	30	31	29
alanine	43	42	44
valine	27	27	28
methionine	4	4	4
isoleucine	22	22	19
leucine	43	44	42
tyrosine	9	11	11
phenylalanine	31	28	31

[a] Expressed as residues/M_r 60 000. [b] DSI used as an abbreviation for disulfide interchange enzyme. [c] Values from Carmichael et al. (1977).

remaining fragments were too small to be detected on polyacrylamide gels. The same pattern of degradation was observed when the enzyme preparations were exposed to very low concentrations of subtilisin. Digestion for 30 min at 37 °C with 0.1% subtilisin (w/w) was sufficient to achieve quantitative cleavage of the enzyme, and the breakdown products were completely inactive as measured by the insulin degradation assay. In view of the susceptibility of the enzyme to proteolysis, the preparations were maintained at -70 °C.

The plasmacytoma and liver enzymes were indistinguishable by all biochemical criteria applied. As shown in Table I, the amino acid contents of the two preparations agreed within the limits of experimental error. The enzymes comigrated on $NaDodSO_4$–polyacrylamide gels with an apparent molecular weight of 60 000 ± 2500 (Figure 1), and they exhibited the same charge distribution on alkaline pH and two-dimensional gels (Figures 1 and 2). In the insulin degradation assay, the specific activities of the preparations were equivalent, 5460 ± 130 and 5470 ± 140 units/mg of protein. The enzymes were also indistinguishable in their immunological properties. The precipitin lines formed in Ouchterlony immunodiffusion fused without spurring (Figure 3B), and the inhibition curves obtained by radioimmunoassay were superimposable (Figure 4). These data indicated that tissues with different specialized functions synthesize the same enzyme.

Distribution of Disulfide Interchange Enzyme in Mouse Lymphoid Tissue. The relationship between the synthesis of disulfide interchange enzyme and Ig secretion was investigated first with normal spleen cells. Cell populations were lysed with nonionic detergent, and the lysates were examined for their ability to inhibit the binding of ^{125}I-labeled liver enzyme to its antibody. Analyses of total spleen populations showed that the average enzyme content was low compared to liver, 1.2 × 10^5 molecules per spleen cell vs. 9.1 × 10^6 molecules per liver cell (Table II). However, examinations of selected spleen populations indicated that the content of disulfide interchange enzyme varied with cell type. Large cell populations, which are known to include most of the lymphocytes differentiated to Ig secretion, were found to have levels of enzyme 3–4 times the average of the population as a whole. On the other hand, small cell populations consisting mostly of resting B and T lymphocytes were relatively depleted of enzyme.

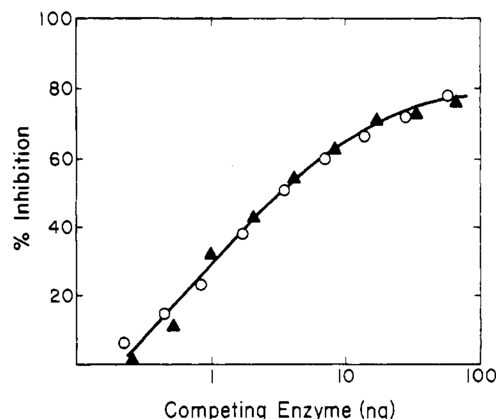

FIGURE 4: Radioimmunoassays of purified disulfide interchange enzyme. Serial dilutions of liver enzyme (○) and plasmacytoma enzyme (▲) were tested for their ability to inhibit the binding of ^{125}I-labeled liver enzyme to anti-liver enzyme.

Table II: Levels of Disulfide Interchange Enzyme in Mouse Lymphoid Cells and Liver Cells

cells	cell type	DSI content: molecules × 10^{-6} per cell	DSI content: total protein[a] (%)
	Normal Tissues		
spleen	all lymphoid elements	0.12	0.07
large spleen cell fraction	enriched for Ig-secreting cells	0.41	0.09
small spleen cell fraction	enriched for non-Ig-secreting T and B cells	0.13	0.03
liver	nonlymphoid	9.1	0.35
	Non-Ig-Secreting Lymphoid Cell Lines		
WEHI 231	B cell lymphoma	0.27	0.02
P1798	thymoma	0.47	0.05
S49	thymoma	0.72	0.08
W7	thymoma	1.4	0.11
	Ig-Secreting Lymphoid Cell Lines		
HPC76	IgM secretor	12	0.40
TEPC 183	IgM secretor	15	0.62
MOPC 104E	IgM secretor	12	0.77
MOPC 315	IgA secretor	3.7	0.36
S194A	IgA secretor	3.2	0.35
Y5606	IgG_3 secretor	17	0.56
P3	IgG_1 secretor	23	0.73
S134	IgG_{2b}	17	0.78
P1	IgG_{2a}	11	0.79

[a] Expressed as percentage of extractable protein that is disulfide interchange enzyme (DSI) as measured by a radioimmunoassay for the enzyme.

For a more precise correlation of the enzyme synthesis with cell type, the radioimmunoassays were extended to transformed lymphoid lines. The results (Table II) clearly showed that the levels of intracellular enzyme depended on the secretory activity of the cells. Lymphoid lines not actively secreting immunoglobulin were found to have a consistently low enzyme content, 3 × 10^5–1.4 × 10^6 molecules per cell. These lines included thymomas representing T cells at various stages of differentiation and a lymphoma representing a resting B cell that expresses monomer IgM on its plasma membrane. In contrast, Ig-secreting plasmacytomas had enzyme levels at least an order of magnitude higher, ranging from 3.2 × 10^6 to 2.3 × 10^7 molecules per cell. The observed increases were real, because similar results were obtained when the enzyme content was determined as the percentage of extractable protein per cell (Table II). The observed increases did not, however,

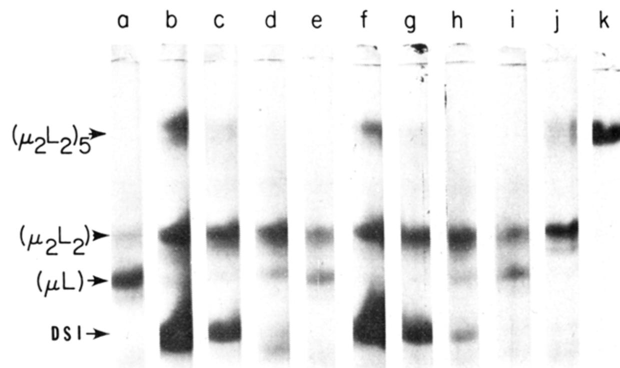

FIGURE 5: IgM polymerizing activity of purified disulfide interchange enzyme. Aliquots containing 25 μg of reduced IgM and J chain were incubated with either (a) buffer, liver enzyme at (b) 2.0, (c) 1.0, (d) 0.5, and (e) 0.25 mg/mL, plasmacytoma enzyme at (f) 2.0, (g) 1.0, (h) 0.5, and (i) 0.25 mg/mL, or (j) 0.1 mg/mL polymerizing component (Roth & Koshland, 1981), and the reaction mixtures were electrophoresed on a $NaDodSO_4$–agarose gel. Pentamer IgM was included as a control in slot k.

Table III: Polymerization of Reduced IgM by Liver and Plasmacytoma Disulfide Interchange Enzyme [a]

conditions (mg/mL)	IgM species [a] (%)				
	L	μ	μL	μ_2L_2	$(\mu_2L_2)_5$
no enzyme	13	22	49	15	0
liver enzyme					
0.25	ND [b]	ND	42	58	0
0.5	ND	ND	20	80	0
1.0	ND	ND	14	65	21
2.0	ND	ND	12	43	45
plasmacytoma enzyme					
0.25	ND	ND	45	55	0
0.5	ND	ND	18	82	0
1.0	ND	ND	9	71	20
2.0	ND	ND	5	56	40

[a] The gels shown in Figure 3 were scanned, and the amounts of light chain (L), heavy chain (μ), disulfide-linked heavy and light chain (μL), IgM monomers (μ_2L_2), and IgM pentamer [$(\mu_2L_2)_5$] were determined and expressed as a percentage of the total IgM species in each gel. [b] The amounts of L and μ chains in gels containing disulfide interchange enzyme were not determined (ND) since the enzyme migrated in the same part of the gel as these 2 IgM species.

correlate with the extent of polymerization of the immunoglobulin product. The enzyme levels in lines secreting monomeric IgG were equivalent to those in lines secreting polymeric IgM or IgA. These results suggested that disulfide interchange enzyme might play a more critical role in the formation of intra- and interchain bonds required for monomer assembly than in the formation of intersubunit bonds required for polymer assembly.

Effect of Disulfide Interchange Enzyme on the Formation of IgM Disulfide Bonds. The role deduced for disulfide interchange enzyme from its distribution in lymphoid tissues was supported by the results of in vitro polymerization assays. The activity of the plasmacytoma and liver enzymes was assessed by incubating increasing concentrations with reduced IgM and monitoring the formation of monomers and pentamers by $NaDodSO_4$–agarose gel electrophoresis. A typical electrophoretic pattern is illustrated in Figure 5, and the quantitation of the products obtained is given in Table III. In the control without enzyme (Figure 5, lane a), more than 80% of the IgM remained in the form of μL dimers or free heavy and light chains and 16% migrated as S–S linked μ_2L_2 monomers. This small percentage of monomer could have resulted from incomplete reduction of the parent pentamer or from spontaneous reassembly during the assay procedure. In the presence of equimolar amounts of enzyme, the amount of reassembled monomer was significantly and specifically increased. Thus, at enzyme concentrations of 0.25 and 0.5 mg/mL, a large proportion of μL, H, and L was converted to μ_2L_2, but there was no polymerization to pentamer (Figure 5, lanes d, e, h, and i). At higher enzyme concentrations, 5–10 molar excess over monomer, pentamer IgM formation could be detected (Figure 5, lanes b, c, f, and g). For 50% polymerization of the IgM molecules, concentrations of enzyme greater than 2 mg/mL were required. In contrast, a distinct polymerizing enzyme isolated from plasmacytoma cells (Roth & Koshland, 1981) effected 50% polymerization at a concentration of 0.1 mg/mL (Figure 5, lane j). These data indicated that disulfide interchange enzyme is capable of catalyzing the formation of intramonomer bonds in IgM but functions only at much higher concentrations, if at all, in the formation of intermonomer bonds.

Discussion

The results of the present studies argue for a single form of thiol:protein disulfide oxidoreductase which is capable of catalyzing both the formation of protein disulfide bonds and the cleavage of insulin disulfide bonds. Hillson & Freedman (1980) have reported that the two enzymatic activities found in bovine liver can be separated by chromatography on thiolpropyl-Sepharose 6B; they have assigned protein disulfide isomerase activity to a component of M_r 57 000 and suggested that glutathione-insulin transhydrogenase activity resides in a component of M_r 37 000. Very different results were obtained in the studies described here of murine thiol oxidoreductase. Preparations isolated from mouse liver and IgM-secreting plasmacytomas showed a single band of M_r 60 000 on $NaDodSO_4$ and two-dimensional gel electrophoresis and were equally efficient at promoting monomer IgM assembly and insulin degradation. Smaller molecular weight species and loss of enzymatic activity were observed only when the purified materials were degraded by proteolytic enzymes. Chromatography of the mouse liver enzyme preparation on thiolpropyl-Sepharose did yield two fractions, but analyses of the fractions showed that each was composed of a 60 000-dalton protein with the same dual enzymatic activity of the parent material (R. Roth, unpublished observations). The homogeneity of the liver and plasmacytoma preparations was supported by immunological analyses. Only a single major component was detected by three different assays, immunodiffusion, immunoelectrophoresis, and inhibitory capacity in a sensitive radioimmunoassay. Although the possibility that the preparations contained very small amounts of a second enzyme cannot be rigorously excluded, the data obtained strongly suggested that a single thiol oxidoreductase mediates both enzymatic activities.

The results of the present studies also argue for a role of disulfide interchange enzyme in the in vivo synthesis of protein disulfide bonds. Assays of lymphoid cells and their transformed counterparts showed that the levels of intracellular enzyme correlate with Ig secretory activity. The enzyme content of cell lines actively secreting Ig was found to be 1–2 orders of magnitude higher than that of a lymphoma line representative of an unstimulated B lymphocyte or of thymomas representative of non-Ig-secreting T lymphocytes. The observed increases in enzyme levels parallel the increases in Ig synthesis that are induced by antigen or mitogen exposures. The unstimulated B lymphocyte has been shown to contain

20 000–50 000 monomer IgM receptors that have a half-life of 12–20 h (Melchers & Anderson, 1973). The Ig-secreting cell, on the other hand, contains on the average 10^6 monomer molecules (Melchers & Anderson, 1973) that are secreted with a transit time of 1–2 h (Choi et al., 1971). It has been calculated from these data that the rate of monomer synthesis is increased 20–100-fold during differentiation to antibody secretion, a value very similar to the changes observed in disulfide interchange enzyme content.

More direct evidence for the physiological function of disulfide interchange enzyme was provided by in vitro polymerization assays. The enzyme was found to catalyze the formation of the interchain bonds that are required for monomer IgM assembly. At equimolar concentrations of enzyme and μL subunits, more than 50% of the μL and free μ and L chains was converted to covalently linked monomers. These results are consistent with information available on in vivo monomer synthesis. Experiments by Bergman & Kuehl (1979a,b) have shown that Ig intra- and interchain bonds are formed very soon after synthesis of the heavy and light chains, even while the polypeptides are attached to the polyribosomes. Disulfide interchange enzyme is in a position to promote these reactions because it is bound to the rough endoplasmic reticulum in close association with the ribosomes (Williams et al., 1968).

Evidence was also obtained from the in vitro polymerization assays that disulfide interchange enzyme does not catalyze the formation of the intersubunit bonds involved in pentamer IgM assembly. At equimolar concentrations of enzyme and monomer subunits, no pentamer product was detected. In 10–20-fold molar excess, the enzyme was found to induce IgM polymerization, but it is unlikely that such activity is of significance in vivo. First, polymer assembly occurs near or at the plasma membrane whereas disulfide interchange enzyme is associated with the rough endoplasmic reticulum. Second, polymer-secreting lymphoid cells have been found to synthesize another enzyme, tentatively identified as a sulfhydryl oxidase, which is associated with the membrane fraction and promotes IgM polymerization at less than one-twentieth the concentration of disulfide interchange enzyme (Roth & Koshland, 1981). Thus, the studies described in this paper indicate that disulfide interchange enzyme performs a physiological role in the formation of the interchain disulfide bonds common to all immunoglobulins but not the intersubunit bonds specific to the polymeric forms.

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