THIOL-DISULFIDE INTERCHANGE IN THE BINDING OF BENCE JONES PROTEINS TO α_1-ANTITRYPSIN, PREALBUMIN, AND ALBUMIN*

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Complexes between light Ig chains of κ-type and α_1-antitrypsin (α_1-AT),[1] prealbumin, and albumin can regularly be detected in plasma from myeloma patients with Bence Jones proteinuria and an urinary excretion of more than 1 g κ-chains per liter urine. These complexes are not found in myeloma with Bence Jones proteinuria of λ-type. The complexes of κ-chains and the mentioned plasma proteins are cleaved by disulfide cleaving reagents in low concentration. Further, a reactive terminal cysteinyl of κ-chains is a prerequisite for formation of the complexes, for which reason a disulfide bond constitutes the reasonable link between the proteins (1, 2).

These complexes are also formed in vitro on mixing of plasma and isolated κ-chains, but the reactions proceed only slowly at physiologic pH. This appears reasonable if an ionized terminal thiol group of a monomeric κ-chain is necessary for a sulfhydryl-disulfide interchange as κ-chains in urine occur mainly as dimers or mixed disulfides (3, 4) and as the pK values of SH groups of peptides and proteins usually are above 8 (5). The complex formation described may also be explained by disulfide interchange between reactive sulfhydryl groups of the specific plasma proteins and mixed disulfides of light κ-chains. This paper reports an attempt to elucidate the reaction between κ-chains and plasma proteins (primarily α_1-AT) and to explain why native λ-chains do not form the corresponding complexes.

Material and Methods

Electrophoresis (6), electroimmunoassay (7), and crossed immunoelectrophoresis (8) were run in 0.8% agarose gel with a 0.075 M barbital buffer of pH 8.6 containing 2mM calcium lactate.

* This investigation was supported by grants from the Swedish Medical Research Council (project no. B75-13X-581-11A) and Alfred Österlunds Stiftelse.

[1] *Abbreviations used in this paper:* α_1-AT, α_1-antitrypsin; BAPNA, benzoyl-DL-arginine-*p*-nitroanilide; CNT, 3-carboxylato-4-nitrothiophenolate; DTNB, 5,5',-dithiobis-2-nitrobenzoate; DTT, dithiothreitol; κ- and λ-chains, immunoglobulin light chain of kappa- and lambda type; CNT-plasma, plasma with CNT-substituted SH groups of plasma proteins; SDS, sodium dodecylsulfate.

Autoradiography was performed with Kodak Trix-Pan film (Eastman Kodak, Rochester, N.Y.). Electrophoresis in sodium dodecylsulfate (SDS)-polyacrylamide gel (7.5 and 10%) was run as described by Weber and Osborn (9). Transferrin, albumin, ovoalbumin, and pepsinogen were used as markers for measurement of molecular size. Before the application the samples were heated (95°C) for 3 min with 0.1% SDS in absence of reducing agents.

5,5',-Dithiobis-2-nitrobenzoate (DTNB) was purchased from British Drug House, dithiothreitol (DTT) from Miles-Seravac, recrystallized trypsin from Novo, Copenhagen. Elastase isolated from dog granulocytes (10) was kindly supplied by Dr. Kjell Ohlsson. Specific antisera against all plasma proteins of concentration above 0.1 g/liter were available at the laboratory. α_1-AT was isolated from human plasma as described recently (11) and albumin was obtained as a by-product during this fractionation.

Native and Derivatized Light Chains. Bence Jones proteins of κ- and λ-type isolated from urines were available as mono- and/or dimers. Monomeric κ-chains as mixed disulfides (CNT-κ-chains) were routinely obtained by incubation of solutions of κ-chains with DTNB (0.01 M) for 16 h in a 0.1 molar Tris-HCl buffer of pH 8.1. Excess DTNB and CNT-ions were separated from the proteins by gel filtration of the solution (1-2 ml) through Sephadex G 25 columns (40 × 0.9 cm) in the same buffer. The protein containing fractions were pooled and concentrated through Diaflo UM 10 filters (Instrument AB Lambda, Stockholm, Sweden).

Monomeric κ-chains were separated from di- and polymers by gel filtration of the solutions (5-10 ml) through Sephadex G 75 columns (85 × 5 cm). Analytical gel electrophoresis was used to find the limits for cut off. Preparative gel electrophoresis (6) was alternatively used for isolation of monomeric CNT-κ-chains utilizing the higher electrophoretic mobility of the CNT-κ-chain complex than of the native κ-chain "monomer" and the κ-chain dimer. Monomeric CNT-λ-chains were prepared in an analogous way. κ- and λ-chains were labeled with ^{125}I using a peroxidase method (12). Plasma with the reactive thiols and disulfides of the proteins derivatized through reaction with DTNB was obtained as described for light chains and was called CNT-plasma.

Formation of Complexes between Light Chains and Plasma Proteins. Pure α_1-AT and albumin were used separately and as mixtures in experiments to measure amounts of complexes formed within 1 day at pH 8.1 on addition of native or CNT-derivatized light chains. The inhibitory effect of DTNB on these reactions was studied on addition of DTNB and CNT-κ-chains in varying ratios to α_1-AT. The complex formation was followed by agarose gel electrophoresis and crossed immunoelectrophoresis with anti-α_1-AT and antialbumin.

Solutions of unfractionated κ-chains, monomeric κ-chains or, of CNT-κ-chains were mixed with plasma (0.01 M Na_2 EDTA) or with CNT-plasma (usually at pH 8.1) and incubated for a varying time. The formation of light chain complexes was followed by crossed immunoelectrophoresis with antisera to specific plasma proteins and to light chains. The rate of complex formation was measured at different pH, in the presence of DTNB in varying concentration and after mild reduction of light chains with DTT.

Formation of Complexes between κ-Chain-α_1-AT and Serine Proteases. The trypsin and elastase binding capacity of plasma and α_1-AT was titrated by addition of increasing amounts of respectively trypsin and elastase and subsequent crossed immunoelectrophoresis with anti-α_1-AT.

About half to one quarter of the α_1-AT of such plasma was converted to κ-complexes by incubation with an equal volume (1-0.4%) of CNT-κ-chain solutions. After 18 h the plasma proteins were separated from the solutions by Sephadex G 25 gel filtration in Tris-HCl pH 8.1. The protein concentration was estimated and trypsin or elastase was added to obtain the 1/4, 1/2, and 3/4 saturation of the α_1-AT with the enzyme, as calculated from the result of the earlier experiments with native plasma. The amount of different α_1-AT complexes formed were estimated by crossed immunoelectrophoresis with anti-α_1-AT. The enzyme inhibition was controlled at pH 8.1 with benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate (13).

Results

The formation of complexes between native κ-chains and specific plasma proteins was completely inhibited in the presence of 0.01 M Ellman's reagent (DTNB). However, the solutions turned yellow indicating thiol exchange reac-

tions with release of 3-carboxylato-4-nitrothiophenolate (CNT) ions. This prompted a study of the complex formation of plasma proteins and/or the light chains reacted with DTNB and formed mixed disulfides with CNT.

Incubation of plasma with DTNB for a day at pH 8.1 and separation of DTNB and CNT-ions from the proteins by a Sephadex G 25 column gave a "CNT-plasma" with the promptly reacting SH-groups and reactive disulfides of specific proteins substituted by CNT. One day's incubation of such plasma with a native κ-chain solution at pH 7.5 yielded κ-complexes in amounts similar to those by native plasma. Addition of a CNT-derivatized κ-chain solution to native plasma gave more complexes than a native κ-chain solution. The results of principle interest in a series of experiments (Table I) prompted more detailed studies on the formation of mixed disulfides of light chains with DTNB and on the interchange reactions of CNT light chains with specific plasma proteins.

Interaction between Light Chains and DTNB. Individual, isolated κ- and λ-chains had a widely varying amount of DTNB reactive thiols. An experiment giving the rate of the DTNB reaction of a pool of 10 κ- and 10 λ-chains respectively is given in Fig. 1. Fewer (6%) of the penultimate SH-groups of the λ-chains

TABLE I

Formation of Light Chain Complexes with Plasma Proteins (α_1-AT, Prealbumin, and Albumin) Before and After One or Both of the Reactants had been Incubated with Ellman's Reagent

Reaction mixtures		Amount of complexes	
		κ	λ
κ-(λ) chains	+ DTNB (0.01 M) + plasma	+	0
κ-(λ) chains	+ CNT-plasma	++	0
κ(λ) chains	+ plasma	++	0
CNT-κ-(λ-)chains	+ CNT-plasma	+++	++
CNT-κ-(λ-)chains	+ plasma	++++	+++

Equal parts of plasma and 1% light chain solutions were incubated for 18 h in Tris-HCl of pH 7.5. The formation of complexes was followed by crossed immunoelectrophoresis with specific antisera.

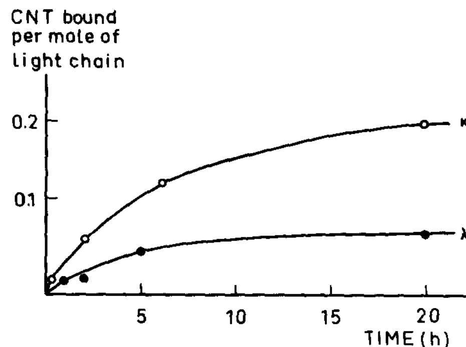


FIG. 1. Formation of mixed disulfides of DTNB and light chains at pH 8.1. The molar fraction formed of CNT-light chain disulfides was calculated from CNT-ions released from the light chains on reduction in relation to amount of light chains occurring in the solution.

reacted within 1 day than of the C-terminal thiols of the κ-chains (18%). The reactive κ-thiols were largely abolished within 1 day. The reaction was highly pH-dependent with strongly decreasing rate below pH 8 as shown for one κ-chain on Fig. 2. The proportions of mono- and dimers of light chains in the solutions were estimated from the intensity of the bands obtained after SDS-polyacrylamide electrophoresis. Comparison of these band patterns with those obtained after agarose gel electrophoresis at pH 8.6 showed that the negative net charge of the light chains increased slightly on reduction, but markedly on formation of mono-

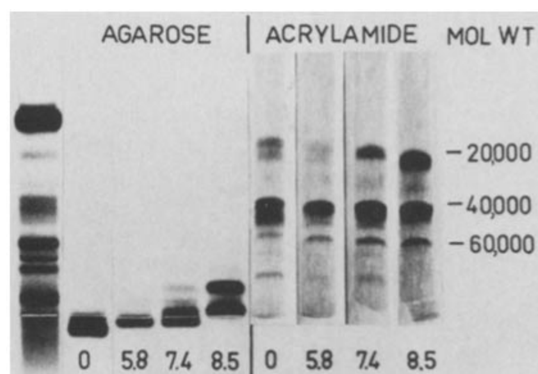


FIG. 2. Effects of DTNB on charge and size of a λ-chain on incubation at different pH. A 2% native λ-chain solution (0) was incubated for 18 h with 0.01 M DTNB at pH 5.8, 7.4 and 8.5. Electrophoretic patterns of the proteins after separation in agarose gel at pH 8.6 to the left (anode at the top). Results of SDS-polyacrylamide gel electrophoresis in the middle and a molecular weight scale as reference to the right. The figures at the bottom indicate the pH of incubation except for the native chain (0).

meric CNT-derivatives and that the ratio monomers to dimers increased on the DTNB treatment (Fig. 3). The monomeric CNT-light chains obtained migrated in agarose anodally of native mono- and dimeric light chains (Figs. 2 and 3). The electrophoretic mobility of the remaining dimers increased only very slightly and much less than the monomers, after incubation with DTNB (Fig. 3).

Interaction between Light Chains, Isolated α_1-AT and Albumin. No evidence of any reaction between α_1-AT and DTNB was obtained by spectrophotometric or electrophoretic analysis. Native and CNT-derivatized light chains were incubated with α_1-AT overnight and analyzed for complex formation by agarose gel and SDS-polyacrylamide electrophoresis. The complexes formed migrated at pH 8.6 in agarose electrophoresis closer to α_1-AT than to the light chains (Fig. 4). The migration in SDS-polyacrylamide (7.5%) for α_1-AT and its κ-chain complex suggested mol wt of 55-60,000 respectively 80-85,000.

Much larger amounts of complexes were obtained with the CNT-derivatized mainly monomeric light chains than with the native chains (Fig. 4). Preceding treatment of α_1-AT with DTNB did not influence on the result. Part of the α_1-AT and the CNT-light chains remained unchanged in the mixture after 18 h. The native κ-chain containing mono- and dimers yielded α_1-AT complexes, but in smaller amount than the monomeric CNT-κ-chain. The native λ-chain occurred

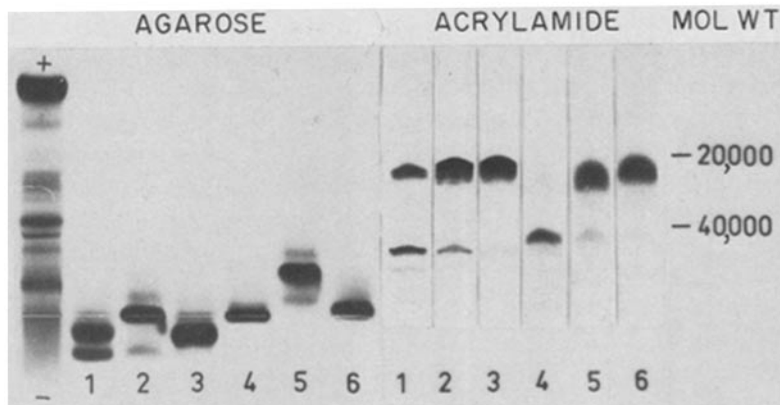


FIG. 3. Effects of DTNB (0.01 M) on charge and size of one κ- and one λ-chain (both about 1%) at pH 8.1 for 18 h and analyzed with agarose gel and SDS-polyacrylamide (10%) electrophoresis. Native κ-chain (1) and λ-chain (4) after 18 h DTNB-reaction (2) and (5). The CNT-light chain disulfides were separated from DTNB and CNT with Sephadex G 25 and reduced with 0.2 M β-mercaptoethanol. The electrophoretic mobility of the reduced κ-chain is given above 3 and the λ-chain above 6.

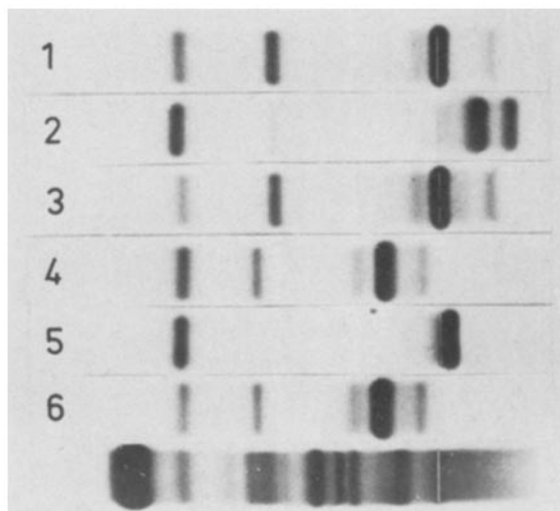


FIG. 4. Complex formation between light chains (native and CNT-disulfides) and α_1-AT (native and DTNB-treated) at pH 7.5 (TRIS-HCl 0.1 M) after incubation for 18 h. (1) DTNB-treated α_1-AT plus CNT-κ-chains; (2) DTNB-treated α_1-AT plus native κ-chains; (3) α_1-AT plus CNT-κ-chains; (4) DTNB-treated α_1-AT plus CNT-λ-chains; (5) DTNB-treated α_1-AT plus native λ-chains; (6) α_1-AT plus CNT-λ-chains.

as a dimer and yielded no complexes, while the mainly monomeric CNT-λ-chain reacted like the corresponding CNT-κ-chain.

Inhibition experiments with DTNB added to α_1-AT before the CNT chains showed that already at molar proportions 1:10 between DTNB and κ-chains produced a slight decrease in amount of complexes. The inhibition was almost total when the ratio was increased to 1.

An isolated monomeric CNT-κ-chain (0.17%) was incubated with α_1-AT (0.23%) respectively albumin (2.1%) overnight at pH 7.5 to compare the amount of reaction products formed. The electrophoretic patterns obtained are given on Fig. 5. About 50% of the CNT-light chain (Fig. 5 E) remained unchanged (Figure 5, nos. 1 and 2) in spite of the large excess of α_1-AT respectively albumin. Addition of the CNT-κ-chains to a mixture of albumin and α_1-AT (Fig. 5, no. 3) yielded more α_1-AT and less albumin complexes. Only trace amounts of the CNT-light chains remained unchanged. In all three experiments were trace amounts of κ-dimers formed (Fig. 5 F).

Interaction between Light Chains and α_1-AT in Plasma. The time-course of the reaction between α_1-AT in native plasma and one CNT-κ-chain is evident from Fig. 6. The amount of complexes formed primarily varied with the monomeric CNT-κ-chain concentration in the mixture. This was supported by the disappearance of the monomeric CNT-κ-chain band on agarose gel electrophoresis concomitant with the formation of complexes. The same holds true for CNT-λ-chain solutions. On addition of CNT-κ-chain solutions to plasma α_1-AT, prealbumin and albumin complexes were formed faster and in larger amounts within one day than on addition of native κ-chains in the pH range 7.4-8.5. Optimal rate of complex formation with α_1-AT occurred about pH 8.

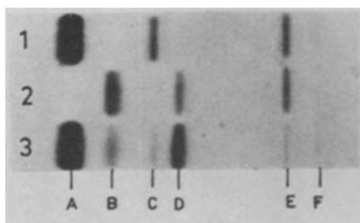


FIG. 5. Complex formation between a CNT-κ-chain (0.17%) and (1) albumin (2.1%), (2) α_1-AT (0.23%), and (3) a mixture of albumin (2.1%) and α_1-AT (0.23%) at pH 7.5 over night. Albumin (A), α_1-AT (B), κ-chain albumin complex (C), κ-chain-α_1-AT complex (D), free monomeric CNT-κ-chain (E), and dimeric κ-chain (F), which are formed in trace amounts mainly in 1 and 3.

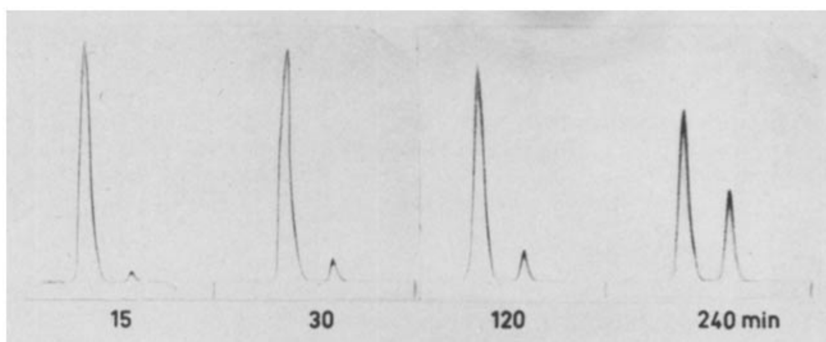


FIG. 6. Time-course of complex formation between α_1-AT and a CNT-κ-chain (pH 8.1) studied by crossed immunoelectrophoresis at pH 8.6 with anti-α_1-AT. One volume of plasma was mixed with one volume CNT-κ-chain (1.2%) and samples drawn for analysis at the intervals given. The degree of complex formation is apparent from the decreasing high α_1-AT peak and the increasing small α_2-peak representing the complexes formed.

The difference in α_1-AT reactivity of native chains and CNT-substituted chains was most marked for λ-chains, which yielded no apparent complexes before treatment with DTNB. The DTNB reactivity of λ-chains varied with formation of 0–30% CNT derivatives within 1 day. Comparative studies of SDS-polyacrylamide light chain patterns and the formation of α_1-AT complexes showed that the λ-chains forming monomers after addition of DTNB also formed plasma protein complexes while those without formation of monomers gave no complexes.

The complex formation of light chain solutions which had freshly been reduced with 0.002 M DTT was no better than the native light chains. All DTT had been separated from the light chains by Sephadex G 25 filtration immediately before its addition to plasma, because residual reducing substance cleaved the complexes efficiently. The time-course for the dimer regeneration after reduction was not studied.

Interaction between Light Chains and the Proteins of Native Plasma. The reaction products obtained between plasma proteins and light chains at pH 7.5 was traced with [^{125}I]CNT-light chains. The reacting plasma proteins were identified with specific antisera and crossed immunoelectrophoresis. The gel electrophoretic patterns and the corresponding autoradiographs are given in Fig. 7 with the κ-chain experiments to the left and the λ-chain to the right. The final concentration of the CNT-κ-chains added was 1.3% and that of CNT-λ-chains 1.1%. Both chains were added as nearly homogenous (Fig. 7, no. 1) monomeric CNT-light chains. They exhibited almost identical complex patterns with prealbumin and albumin, but the κ-chain converted some 90% of the α_1-AT to complex, while the λ-chain converted only about 50%. Both chains formed five prealbumin complexes with similar relative concentration (Fig. 7, no. 5) and only some 15% of the prealbumin remained in free form. Albumin formed a complex that appeared as a distinct fast α_2-band on the gel electrophoretic patterns constituting about 10% of the albumin fraction (Fig. 7, no. 4). The autoradiograph of the gel electrophoresis for plasma plus [^{125}I]CNT-κ-chains (Fig. 7, no. 3) showed the albumin and α_1-AT complexes in the α_2-zone. Residual CNT-κ-chains are seen in front of the slit and, on the cathodal side, free light chains (Fig. 7, no. 3, 4) which did not occur in the solution added (Fig. 7, no. 1). The corresponding λ-chain pattern gave essentially the same information, but the tendency of the chain to form complexes was not so strong as that of the κ-chain. The autoradiographic patterns indicated (Fig. 7, no. 3) that none of the major plasma proteins had any tendency to form complexes other than the three studied earlier.

Trypsin and Elastase Interaction with α_1-AT-Light Chain Complexes. The trypsin-binding capacity of α_1-AT in plasma was estimated in the absence and presence of its κ-chain complexes. Plasma was enriched with CNT-κ-chains so that the ratio between free α_1-AT and κ-chain-α_1-AT was about 4:5 (Fig. 8, no. 0). Trypsin was added to saturate the trypsin-binding capacity of plasma to 25, 50, and 75%, respectively. Crossed immunoelectrophoresis was run with antiserum against α_1-AT to estimate the amount of complexes formed. Some results are given in Fig. 8. The free α_1-AT (1) and the κ-chain-α_1-AT complex (2) gave slightly higher peak heights of their antibody complexes per unit α_1-AT than

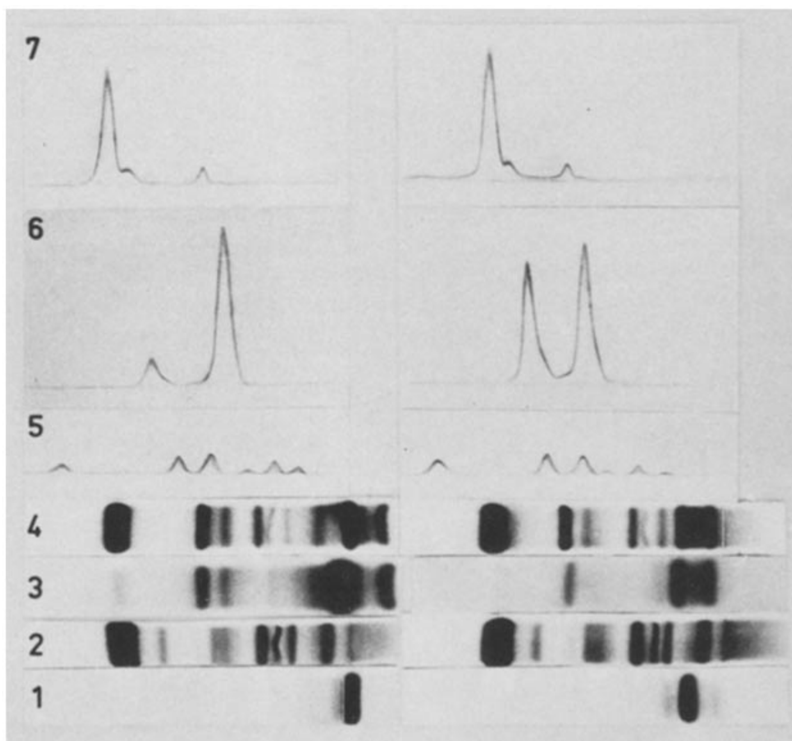


FIG. 7. Complex formation between plasma proteins and κ-chains (left half) and λ-chains (right half). CNT-light chain mixed disulfides (gel electrophoresis 1) was added to EDTA-plasma (2) and incubated for 18 h before agarose gel electrophoresis (4) at pH 8.6. The light chains were tagged with ^{125}I and the pattern of (4) was also developed by autoradiography (3). Crossed immunoelectrophoresis of (4) was run with antisera against prealbumin (5), α_1-AT (6) and albumin (7). Crossed immunoelectrophoresis of plasma (2) before addition of the light chains yielded single peaks corresponding to free albumin, α_1-AT and prealbumin. The cathodal asymmetri of the peak on 7 for free albumin originates from dimers. The ratio between free protein and complex in 5, 6, and 7 can be estimated from the ratios between the areas enclosed by the precipitation lines. Five complexes are recognized for prealbumin (5) while α_1-AT and albumin formed each one complex. These two complexes are recognized as bands in the α-zone on 4. The intensely stained band of the albumin complex on 4 corresponds to a small peak on 7 since the complexes constituted only about 10% of the albumin.

their trypsin complexes (3, 4) because of their lower charge. Trypsin formed complexes as readily with α_1-AT as with κ-chain-α_1-AT complexes. Both trypsin complexes were slightly heterogenous with anodally skew peaks. The trypsin of both complexes was inactivated since the BAPNA splitting activity of the trypsin was lost in the three samples.

The corresponding interaction experiments performed with dog leukocytal elastase and α_1-AT respectively κ-chain-α_1-AT gave results in agreement with the trypsin experiments. SDS-polyacrylamide electrophoresis suggested a mol wt of about 25,000 for elastase and about 75,000 for the α_1-AT complex, while the elastase-κ-chain-α_1-AT complex dissociated in SDS into one 75,000 and one 25,000 unit.

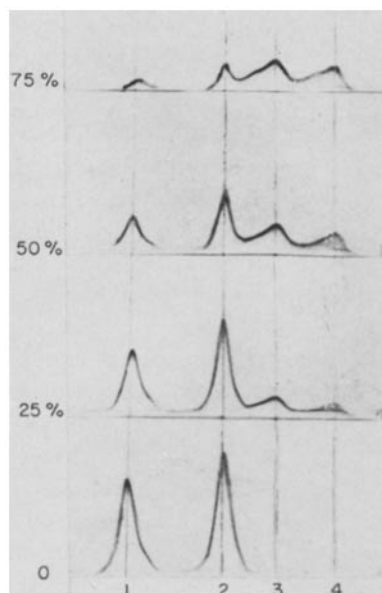


FIG. 8. Crossed immunoelectrophoretic patterns showing the partition of trypsin between α_1-AT and its κ-chain complex in plasma on stepwise addition of trypsin (barbital buffer pH 8.6, antiserum to α_1-AT). The plasma (0) contained slightly more than 50% of the α_1-AT (1) as a κ-chain complex (2). The trypsin binding capacity of plasma was saturated to 25, 50, and 75% by addition of trypsin. The complexes between trypsin and α_1-AT are recognized above 3 and between trypsin and κ-chain-α_1-AT complexes above 4.

Discussion

Light Chain Reactions with DTNB. Bence Jones proteins from urine occur as mixtures of monomers with blocked C-terminal sulfhydryl groups and as noncovalently or disulfide linked dimers. The λ-type has usually less monomers and more disulfide linked dimers that the κ-chains (3, 4). A single, carefully studied κ-chain with blocked C-terminal interchain sulfhydryl group was proven to occur at physiologic pH as a monomer-dimer system with an association constant of 10^5 liters/mol (14). Robyt et al. (15) have proved that DTNB reacts not only with thiolate ions at pH 8 as generally accepted but also with disulfides through CNT-ions released in the presence of catalytic amounts of sulfhydryl groups. Addition of DTNB to light chain solutions gave partial monomerization through formation of CNT-derivatized light chains. The κ-chains usually gave a greater monomeric fraction than the λ-chains, which sometimes gave no monomers. This suggests that the λ-chain disulfides are less reactive to CNT than the noncovalent κ-chain dimers are to DTNB. The charge difference between native monomers, dimers, and the CNT derivatives is sufficient to follow the DTNB reactions by simple agarose gel electrophoresis. The C-terminal position of cystein in κ-chains and their penultimate position of λ-chains may contribute to the higher DTNB-reactivity of κ-chains. In view of the constant sequence of the C-terminal part of the light chains the difference found in reactivity between light chains of identical type was unexpected. The different stability of the light chain dimers may influence their tubular reabsorption and catabolism and may be related to the unpredictable development of renal lesions in Bence Jones proteinuria. Gel electrophoresis of Bence Jones proteins before and after DTNB-treatment offers a simple way to

measure the dimer-monomer transition. The molecular size influences probably the relation between the tubular catabolism and the urinary elimination of light chains.

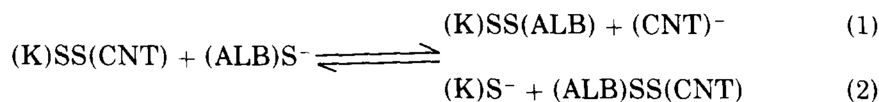
On the Different Tendency of κ- and λ-Chains to form Plasma Protein Complexes. A minor, monomeric fraction of native κ-chains formed complexes relatively slowly with α_1-AT, prealbumin, and albumin in plasma (2) for which reason it was not clear whether the probable sulfhydryl-disulfide interchange causing the complexation occurred between a free κ-chain of short half life and/or its mixed disulfide (e.g. with cystein) and the plasma proteins. Mixed disulfides of light chains (CNT-$\kappa(\lambda)$-chains) gave much more complexes than native or freshly reduced light chains. The difference was most striking for λ-chains, which normally formed no complexes with the plasma proteins. After conversion to monomeric CNT-λ-chains they showed an even stronger tendency to form complexes than the native κ-chains and similar to CNT-κ-chains. Monomerization of the light chains precedes the formation of plasma protein complexes and the lower dimer stability of κ- than of λ-chains principally explains why much more κ- than λ-chain-plasma protein complexes are found in Bence Jones proteinemia.

Remaining DTNB in the light chain solution on its addition to plasma inhibited the formation of plasma protein light chain complexes. This further supports that sulfhydryl-disulfide interchange causes the formation of the protein-protein linkage, since DTNB serves as a trap of free sulfhydryl groups.

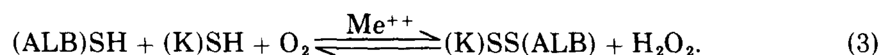
Interaction of Pure α_1-AT and Light Chains. The two half cysteins of α_1-AT probably occur as a disulfide bridge since they do not react with DTNB in 6 molar guanidin HCl or 3 molar guanidine HCl and 6.5 molar isopropanol (11). Interchange of this disulfide with thiol groups of other proteins is supported by our recent finding of the regular occurrence in plasma, of an IgA fraction linked to α_1-AT by a disulfide bond (2). From the results reported above it may be concluded that the addition of monomeric CNT-derivatized light chains to pure α_1-AT yielded more complexes than did native monomers mainly occurring as mixed disulfides with cystein. The SDS-acrylamide electrophoresis indicated formation of covalently linked complexes (1:1). This might support interchange between a hidden SH-group of α_1-AT and the mixed disulfide. Spontaneous cleavage of the mixed disulfides with release of highly reactive light chain monomers attacking an -S-S-bridge of α_1-AT in absence of DTNB may be offered as an alternative explanation. The complex formation was inhibited by DTNB in low concentration even though it did not react with α_1-AT. This lends support to the assumption of an interchange reaction between monomeric κ-chain thiolate ions in statu nascendi and the disulfide in α_1-AT on the cleavage of the bridge. This hypothesis was supported by the finding that more light chain-α_1-AT complexes were formed when albumin was added to the mixture (Fig. 5, no. 3). The thiol group of albumin interchanged with CNT-light chains and linked preferably CNT with release of monomeric, reactive light chains, which apparently had a stronger tendency to interchange with the -S-S-bridge of α_1-AT than to form -S-S-homologous interchain-dimers since only traces of dimers were generated. The presence of α_1-AT decreased the amount of κ-chain-albumin complexes. Both these findings support the view that α_1-AT has a strong affinity for monomeric light chain thiolate ions.

The association of one κ-chain to one α_1-AT did not influence on its high affinity for trypsin or elastase as judged from the partition of these enzymes between α_1-AT and its κ-chain complex (Fig. 8). The stability of these two types of protease complexes has not been compared, but the findings of the linkage and the inhibition of the serine proteases indicate that the firm linkage mainly concerns other parts of the α_1-AT molecule than the cysteinyls. Thus, an intrachain disulfide bridge is not necessary for the protease inhibitory function of α_1-AT. This contrasts with the structural model shown for most trypsin inhibitors (17).

Light Chain Complexes Formed in Plasma. The amount of light chain protein (prealbumin, α_1-AT and albumin) complexes formed within one day in mixtures of different light chains and plasma varied mainly with the amount of CNT-light chain monomers added. This suggested a disulfide interchange with more or less reactive -S-S- or SH-groups of the mentioned plasma proteins. Each of the four subunits of prealbumin has one SH-group (16) and albumin has 0.6-0.8 thiols per mole (18) besides its 17 intra-chain-S-S-bridges. This gives about 20 times more albumin than prealbumin thiols. Probable reactions behind the formation of albumin complexes are as follows since the complex formation proceeds faster at pH 8 than at 7 and the thiolate ion is more nucleophilic than the SH group:



$$\text{(K)SS(CNT)} + \text{(ALB)S}^- \rightleftharpoons \begin{cases} \text{(K)SS(ALB)} + \text{(CNT)}^- & (1) \\ \text{(K)S}^- + \text{(ALB)SS(CNT)} & (2) \end{cases}$$



$$\text{(ALB)SH} + \text{(K)SH} + O_2 \overset{Me^{++}}{\rightleftharpoons} \text{(K)SS(ALB)} + H_2O_2. \qquad (3)$$

The κ-chain-albumin complexes obtained lend support to the assumption of the reactions (1) and/or (3). The latter is less probable as this oxidation proceeds smoothly only in the presence of catalyzing metal ions (Me^{++}), but the formation occurred in the presence of EDTA. The alternative (2) is strongly supported by the appearance of free light chains during the reaction (Fig. 7, no. 3 and 4 and Fig. 4, nos. 1, 3, 4 and 6) and from the model experiments with mixtures of α_1-AT, κ-chains, and albumin. It is thus apparent that albumin causes release of reactive light chain monomers by reaction (2) for interchange with the α_1-AT disulfide which is in agreement with the findings presented on Fig. 5, no. 3. These reactive light chains may also be an alternative of the CNT-light chain as reaction partner for prealbumin whose four thiols are not accessible to DTNB except in hydrophobic environment (16).

The similarity in pattern between plasma protein light chain complexes formed with CNT-κ-chains and native κ-chains suggested that the reactive constituent of native κ-chain solutions is the monomeric cystein-derivatized κ-chains. The monomeric fraction but not the dimers disappeared with the formation of complexes. No complexes occur in plasma between any of albumin, prealbumin, and α_1-AT in spite of the reactive thiols or disulfides while both albumin and α_1-AT form IgA complexes as a result of thiol-disulfide interchange (19). This suggests steric or local charge hindrance around the thiols for their interaction. There is little reason to believe that the interaction between these three proteins and light chains of either type is unique. It is more probable that in plasma and intercellular fluids such interactions occur regularly between peptides containing readily available disulfides or thiols and these carrier proteins of plasma. The

interchange reactions induced in vitro yielded the complexes formed in vivo. In view of the plasma concentration of the specific proteins discussed it is apparent from Fig. 7 that the reactivity of prealbumin and α_1-AT for κ-chains is similar and at least 10 times higher than that of albumin.

The CNT-κ-chain reacted faster with α_1-AT than the CNT-λ-chains (Fig. 7, no. 6) but no difference in reactivity to albumin or to prealbumin was found between the two chains (Fig. 7, no. 5 and 7). These findings support the assumption that the C-terminal serine of λ-chains slightly decreases the reactivity of the cysteinyl to the disulfide bridge of α_1-AT, but has no influence on the reaction with prealbumin and albumin.

Experiments with [^{125}I]tagged light chains excluded the possibility of other plasma proteins having any capacity of quantitative importance for the thiol-disulfide interchange of plasma. This type of interchange reaction may be of some importance for the inactivation and transport of "toxic" peptides released during proteolysis into the extracellular space. The cleavage of the complexes by mild reduction is in line with the assumption of a transport function of the plasma proteins.

Summary

Native light Ig chains of κ- but not of λ-type form -S-S-linked complexes with prealbumin, α_1-AT and albumin in vivo. κ-chains isolated from urines have cysteinyls which are more promptly reacting with dithionitrobenzoate (DTNB) than λ-chains. Both are monomerized on this reaction. On addition to plasma mixed disulfides between both types of light chains and DTNB form larger amounts of complexes than the native chains. The lower reactivity of native λ-chains than of κ-chains to the plasma proteins can be explained by their higher dimer stability.

From the light chain reactions obtained with isolated α_1-AT and albumin it is concluded that α_1-AT has a disulfide which efficiently interchanges with monomeric, light chain thiolate ions released from thionitrobenzoate derivates of light chains and that on interchange with the derivatized light chains albumin releases more free light chains into the solution than are bound to albumin. Addition of derivatized light chains to a mixture of α_1-AT and albumin increases the yield of α_1-AT complexes and decreases the amount of albumin complexes formed. The relative amount of the different complexes formed in the latter experiments corresponds to the findings in vivo in patients with Bence Jones proteinemia.

Prealbumin and α_1-AT in plasma have a roughly 10-fold stronger tendency to link the light chains than albumin. The complexes are formed through thiol-disulfide interchange though neither the disulfide of native α_1-AT nor the thiols of prealbumin is available for reaction with DTNB. The three plasma proteins may together constitute a system for linkage and transport of peptides with reactive thiols or disulfides released into the extracellular fluids.

The trypsin and elastase binding and inhibiting capacity of α_1-AT remains after cleavage of the internal -S-S-bridge of α_1-AT through interchange with a

light chain thiol for which reason an intact internal -S-S-bridge of α_1-AT is not necessary for inhibition and linkage of the enzymes.

Received for publication 30 October 1974.

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