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Structure of Immunoglobulin A. I. Interchain Disulfide Bridges of a γA1 Myeloma Protein*

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ABSTRACT: A γA1 κ-light-chain-type myeloma protein was partially reduced and alkylated with [^{14}C]iodoacetic acid in order to study the interchain disulfide bridges. After digestion, six carboxymethylcysteine peptides were obtained (α1 to α6). One of them (α1) was shown to bridge heavy to light chains and its sequence and function was similar to a peptide obtained from a protein belonging to the Am2 (−) genetic variant of γA2-globulins. A second peptide (α2) rich in proline, cysteine, and carbohydrate probably represented the "hinge" region. A third one (α6) was at the C-terminal end of the heavy chain. The location and function of the other three carboxymethylcysteine peptides are not known.

In recent years, immunoglobulin A (IgA) has assumed great significance because although it is a minor constituent of serum it is the predominant immunoglobulin present in external secretions derived from mucosal surfaces, and thus appears to play an important role in local defense mechanisms of the body (Tomasi and Bienenstock, 1968). Like other immunoglobulins, serum IgA appears to consist of four polypeptide chains, two light chains which are common to all immunoglobulins, and two heavy chains (α) which determine the properties characteristic of this class. Two antigenically distinguishable subclasses known as γA1 and γA2 have been recognized (Feinstein and Franklin, 1966; Kunkel and Prendergast, 1966; Vaerman and Heremans, 1966). The antigenic differences are located on the α chains and are independent of light-chain class and monomer–polymer-related structures. In 1968, Grey *et al.* reported that the γA2-immunoglobulins lacked the disulfide bond linking the heavy and light chains in all other classes of immunoglobulins. Instead, the γA2 fraction consists of a pair of disulfide-bonded light chains which are bound to the α chains by noncovalent bonds. More recently, Kunkel *et al.* (1969) and Vyas and Fudenberg (1969) described a genetic marker associated with the γA2 fraction which was called Am2 to conform with the terminology used in the Gm system for γG-globulins. Family studies showed evidence for close linkage of the Am2 marker to the Gm system (Kunkel *et al.*, 1969). Jerry *et al.* (1970) reported that the γA2-immunoglobulins belonging to the genetic variant Am2 (+) were responsible for the previously noted behavior of the γA2 fraction since they were the predominant component and dissociated in the presence of acid or urea into heavy- and light-chain dimers without reduction of the disulfide bonds. In contrast, the rarer Am2 (−) fraction resembled the other immunoglobulins in having the light and heavy chains linked by a single disulfide bond.

IgA present in secretions has a higher molecular weight than serum IgA since it exists as a dimer or trimer linked to an additional polypeptide chain called secretory piece or transport piece, having a molecular weight of approximately 60,000 (Tomasi and Bienenstock, 1968). A new component called J chain present in rabbit and human secretory IgA, as well as in dimeric forms of human IgA myelomas, has been described recently (Halpern and Koshland, 1970; Mestecky *et al.*, 1971). It has been suggested that the J chain might be involved in maintaining the tertiary structure of the polymeric immunoglobulin molecule.

Prior studies of the four subclasses of γG-globulins have clearly demonstrated the value of characterizing the intrachain

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and interchain disulfide bridges in order to elucidate some of the structural features characteristic of the various immunoglobulins. (Frangione *et al.*, 1969a,b). In particular, in the four subclasses of γ chains, the intrachain disulfide bridges were strikingly similar, while the interchain disulfide bridges differed both in number and in position and thus proved to be unique and characteristic for each subclass. Based on this prior experience, we subjected a γA1 κ myeloma protein to mild reduction so as to selectively cleave and characterize the interchain disulfide bridges. In addition, because of the presence of a disulfide bridge linking the heavy and light chain in the Am2 (−) γA2 myeloma proteins we also isolated and sequenced the cysteine-containing peptide which binds the heavy chain to the light chain in an Am2 (−) γA2 myeloma protein.

Materials and Methods

Two γAk myeloma proteins were studied in detail and a third one was subjected only to preliminary studies. Two of these, Pat and Oso were of the γA1 type while the other, Rou, was γA2, Am2 (−). The myeloma proteins were isolated from sera by starch zone electrophoresis at pH 8.6 (Kunkel, 1954) followed by gel filtration on Sephadex G-200 in 0.3 M saline, and tested for purity by immunoelectrophoretic analysis using rabbit antisera to whole human sera, and to γG-immunoglobulins. They were further classified as IgA1 and IgA2 with antisera specific for α_1 and α_2 determinants. The anti-α_1 serum was prepared in rabbits while the anti-α_2 serum was obtained in a baboon through the courtesy of Dr. J. Moor-Jankowski, Primate Laboratory, Sterling Forest, N. Y. The antisera were made specific by absorption with umbilical cord serum and a pool of IgA2 or IgA1 myeloma proteins, respectively (Feinstein and Franklin, 1966).

Partial Reduction and Radioactive Alkylation. Protein Pat was dissolved at a protein concentration of 20 mg/ml in 0.27 M Tris·HCl buffer (pH 8.2). The protein was partially reduced with 0.01 M dithiothreitol at room temperature under N_2. After 1 hr, reduction was terminated by the addition of [^{14}C]-iodoacetic acid (0.02 M, specific activity 0.7 mCi/mmole). The reaction was allowed to proceed for 1 hr at room temperature following which the mixture was dialyzed against water and subsequently 1 M acetic acid. Heavy and light chains were separated by chromatography on Sephadex G-100 in 1 M acetic acid (column 3 × 130 cm). The column effluent was monitored by absorbance of the fractions at 280 mμ. The heavy- and light-chain pools were freeze-dried and tested for purity by immunoelectrophoretic analysis using rabbit antisera against α and κ chains.

Enzyme Digestions and Separations of Peptides. The labeled heavy chain (300 mg) was digested with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone trypsin (Worthington) in 0.2 M ammonium bicarbonate (pH 8.3) for 15 hr at 37°, enzyme–substrate ratio 1:50 (w/w). The digest was freeze-dried and dissolved in 5 ml of 1 M acetic acid. The soluble peptides were separated by chromatography on a column of Sephadex G-50 (3 × 130 cm) equilibrated in 1 M acetic acid at room temperature. Fractions of 5–6 ml were collected at a flow rate of 25 ml/hr. The eluates were monitored by measuring the radioactivity on 50-μl aliquots dissolved in a toluene-based counting solution containing 10% (v/v) Bio-Solv 3 (Beckman) and counted in a Beckman LS-150 liquid scintillation counter.

The insoluble material, as well as some of the soluble peptides, were further digested with pepsin (Worthington, twice crystallized), enzyme–substrate ratio 1:50 (w/w), in 5% formic acid for 15 hr at 37°.

Purification of Radioactive Peptides. The first step in purification was high-voltage electrophoresis on Whatman No. 3MM paper at pH 3.5. The major radioactive peptides were then cut out and oxidized with performic acid vapor (50 ml of formic acid plus 2.5 ml of 30% H_2O_2) for 3 hr in a desiccator at room temperature, to change the charge of CM-cysteine peptides, thus allowing further purification by reelectrophoresis at pH 3.5. The peptides were then subjected to high-voltage electrophoresis at pH 6.5 and 2.1. The following buffers were used: pH 3.5 (pyridine–acetic acid–water, 1:10:190, v/v), pH 6.5 (pyridine–acetic acid–water, 33:1:300, v/v), and pH 2.1 (formic acid–acetic acid–water, 1:4:45, v/v). The radioactive peptides were localized by autoradiography, using Kodak Royal Blue Medical X-ray film. Mobilities at pH 6.5 are expressed as fractions of the distance between ϵ-DNP-lysine and aspartic acid (Offord, 1966).

Amino Acid Analysis. Peptides were hydrolyzed at 110° for 20 hr with 6 N HCl containing 0.1% phenol, in evacuated and sealed tubes. Quantitative amino acid analyses were performed on a Beckman Model 121 automatic amino acid analyzer equipped with high-sensitivity cuvets and recorder. Values are expressed relative to one of the residues taken as 1.0. Amino sugars were qualitatively recognized on the short column of the amino acid analyzer.

Determination of Amino Acid Sequences. N-Terminal residues were identified with dansyl chloride (Gray, 1967) and the derivatives characterized as described by Woods and Wang (1967). Serine and threonine, or glutamic acid and aspartic acid, were identified with solvent IV of Crowshaw *et al.* (1967). Edman degradation was done manually (Frangione and Milstein, 1968) using dansylation to mark the new amino-terminal residues. The dansyl derivative of carboxymethylcysteine cannot be detected with the solvents used because it does not separate from DNS-OH. Therefore, when no dansyl-amino acid was found the presence of carboxymethylcysteine was confirmed by the decrease in negative charge of the peptide after Edman degradation or by the decrease in radioactivity. The results of the dansyl-Edman procedure are shown with arrows under the peptide. Carboxypeptidase B (diisopropyl phosphorofluoridate treated, Worthington) digestion was carried out in 0.1 M ammonium bicarbonate for 12 hr at 37° using 5 μg of enzyme/0.01 μmole of peptide. The digest was freeze-dried and applied to the amino acid analyzer.

Cystine Diagonal "Maps." These were carried out at pH 3.5 by the method of Brown and Hartley (1966) on peptic-tryptic digests of whole IgA protein.

Results

Figure 1 shows the separation of the soluble tryptic peptides obtained from the heavy chain of protein Pat (γA1) on a Sephadex G-50 column. The column fractions were combined as shown in the figure and four fractions were studied in detail. Pools I and II were further digested with pepsin. The amino acid compositions, without corrections, of the major radioactive peptides isolated from each of the peaks are shown in Table I and the amino acid sequences and possible functions are listed in Table II.

Peptide TPα2. This peptide had a high content of proline and cysteine, two features characteristic of the hinge region in the γG-globulins. It also contained carbohydrate detected on the amino acid analyzer. The dansyl-Edman procedure gave its N-terminal sequence, and digestion with carboxypep-

TABLE I: Amino Acid Composition of Carboxymethylcysteine Peptides Obtained from Partial Reduction and Alkylation of Protein PAT (γA1).[a]

	Peak				Precipitate				
	I	II	III	IV					
Peptide[b]	TPα2[c]	TPα5	Tα3	Tα4	TPα1	YPα1a	TPα6	TPα6a	TPα3
Lys			1.07	1.12					
His	1.01		0.98						
Arg	1.00								
CM-CysSO_2	2.34	0.61	1.34	0.65	0.72	0.76	0.83	0.61	1.53
Asp			1.74		2.13	2.00	1.00	1.00	1.02
Thr	4.82	1.87		2.72	1.00	0.85	1.04	0.85	
Ser	5.00		2.50	1.06	1.95	1.14			
Glu			1.06	1.06	1.03	1.08	1.01	1.00	
Pro	11.20		1.71	1.05	0.82	0.88			
Gly			2.82		1.02	1.19	1.21	1.12	1.00
Ala			1.00	1.96			0.97	0.83	
Val	2.59		1.85				1.08	0.75	
Leu		1.00	1.65		1.00				1.12
Tyr			0.54	0.51			0.58		
Phe				1.00					
CHO**[d]	+								
Mobilities at pH 6.5	0.15	0.50	0.40	0.20	0.50	0.60	0.77	0.85	1.00

[a] Compositions are reported as moles of amino acid per mole of peptide. [b] Hydrolysis for 20 hr. [c] TP = trypsin–pepsin; T = trypsin. [d] Detected on the amino acid analyzer.

tidase B yielded Arg as the C-terminal residue. The partial amino acid sequence of this peptide is: Val-Thr-Val-Pro-Cys-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-(Thr,-Pro_4,Ser_2,Cys_2,His)Arg.

The presence of three residues of valine is in agreement with the value of 2.6 obtained by amino acid analysis, since in 20 hr hydrolysis the peptide bonds in which valine is involved are not completely hydrolyzed. Assuming 80% of recovery of carboxymethylcysteine sulfone after 20 hr hydrolysis, the value of 2.3 residues obtained represents three residues present in the peptide.

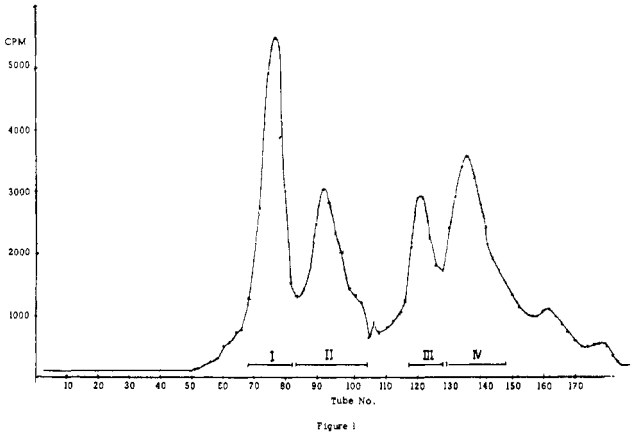

FIGURE 1: Gel filtration of a tryptic digest of partially reduced and [^{14}C]carboxymethylated heavy-chain protein (Pat, γA1) on Sephadex G-50. Peptides from 300 mg of protein were dissolved in 5 ml of M acetic acid, the precipitate was separated and the solution was added to the column (3 × 130 cm) equilibrated in the same solvent. Fractions of 5–6 ml were collected at a flow rate of 25 ml/hr. Radioactivity was measured on 50-μl aliquots. The pooled fractions are indicated on the figure.

Due to the high content of proline in this peptide some proline was carried over in several steps of the dansyl-Edman procedure. Therefore the residual peptide was hydrolyzed at certain steps. Table III shows that the results after the 6th, 11th, and 17th steps were in agreement with those given by the dansyl-Edman procedure. The peptide was submitted to electrophoresis at pH 6.5 after each step up to the 18th. The only change in mobility was observed when the fifth amino acid was removed. The mobility changed from 0.15 to neutrality, and remained neutral after 18 amino acids were removed, thus showing that no other carboxymethylcysteine was present between positions 6 and 18. It was not possible to establish the position of the carbohydrate in the peptide.

Peptide TPα5. The sequence of this peptide was established by the dansyl-Edman procedure as: Thr-Cys-Thr-Leu.

Peptide Tα3. The dansyl-Edman procedure gave the partial sequence: Asp-Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser (Asp, Ser,Glu,Pro_2,Gly_2,Ala,Val,Leu,His)Lys. This peptide presumably included peptide TPα3 obtained from the precipitate, which had the sequence: Asp-Leu-Cys-Gly-Cys but the possibility that the same sequence is repeated in another region of the molecule cannot be excluded.

Peptide Tα4. The sequence of this peptide was established by the dansyl-Edman procedure to be as follows: Thr-Phe-Thr-Cys-Thr-Ala-Ala-Tyr-Pro-Glu-Ser-Lys.

The presence of a glutamic acid residue rather than glutamine is based on the mobility of the peptide (0.2 at pH 6.5) which indicates a net charge of −1.

Peptide TPα6 and TPα6a. These two peptides, obtained after peptic digestion of the precipitate, have the same amino acid composition and differ only in the presence of an extra

TABLE II: Sequence and Function of the Carboxymethylcysteine Peptides Obtained after Partial Reduction and Alkylation of Protein Pat (γA1).

Peptide[a]	Sequence	Function
α1	Ser-Leu-Cys-Ser-Thr-Glx-Pro-Asx-Gly-Asx	H–L
α2	Val-Thr-Val-Pro-Cys-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro	H–H
	Ser-Pro-Ser-Thr(Thr,Pro_4,Ser_2,Cys_2,His)Arg	
α3	Asp-Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser(Asp,Ser,Glu,Pro_2,Gly_2,Ala,Val,Leu,His)Lys	?
α4	Thr-Phe-Thr-Cys-Thr-Ala-Ala-Tyr-Pro-Glu-Ser-Lys	?
α5	Thr-Cys-Thr-Leu	?
α6	Ala-Glu-Val-Asp-Gly-Thr-Cys-Tyr	C terminal
α6a	Ala-Glu-Val-Asp-Glu-Thr-Cys	

[a] See Table I.

TABLE III: Amino Acid Analyses of Peptide TPα2 at Several Steps of the Edman Degradation.[a]

Amino Acid[b]	His	Arg	CM-Cys	Thr	Ser	Pro	Val
Initial	1.0 (1)	1.0 (1)	2.3 (3)	4.8 (5)	5.0 (5)	11.2 (11)	2.6 (3)
Edman 6th step	0.8 (1)	1.0 (1)	1.3 (2)	4.0 (4)	5.0 (5)	8.8 (9)	0.5 (1)
Edman 11th step	0.8 (1)	1.0 (1)	1.3 (2)	2.5 (3)	4.4 (4)	7.3 (7)	0 (0)
Edman 17th step	0.7 (1)	1.0 (1)	1.3 (2)	1.5 (2)	2.3 (2)	3.8 (4)	0 (0)

[a] The theroetical integral value is given in parentheses. [b] Hydrolysis for 20 hr. Compositions are reported as moles of amino acid per mole of peptide.

TABLE IV: Comparison of the Peptide Containing the Heavy–Light Disulfide Bridges of γA1 and a Genetic Variant of γA2-Globulins with Those of Other Immunoglobulins.

Chain	Sequence	Reference
α1	Ser-Leu-Cys-Ser-Thr-Glx-Pro-Asx-Gly-Asx	
α2, Am2(−)	Cys-Ser-Thr-Glx-Pro-Asx-(Gly,Asp)	
μ	Val-Ser-Cys-Glx-Asx-Ser(Thr,Ser,Pro,Asp)	Pink and Milstein (1967), Wikler *et al.* (1969) Frangione *et al.* (1971)
γ4	Ala-Pro-Cys-Ser-Arg-Ser-Thr-Ser-Glu-Ser	Frangione *et al.* (1969a)
γ3	Ala-Pro-Cys-Ser-Arg-Ser-Thr-Ser-Gly-Gly	Frangione *et al.* (1969a)
γ2	Ala-Pro-Cys-Ser-Arg-Ser-Thr-Ser-Glu-Ser	Frangione *et al.* (1969a)
γ1	Ala-Pro-Ser-Ser-Lys-Ser-Thr-Ser-Gly-Gly	Press and Hogg (1969), Edelman *et al.* (1969)

tyrosine in Tα6. The sequence of the peptide TPα6a, as given by the dansyl-Edman procedure is: Ala-Glu-Val-Asp-Gly-Thr-Cys. The mobility of 0.85 at pH 6.5 is in agreement with a net charge of −3 for this peptide, thus showing the presence of aspartic and glutamic acid rather than their amides. These peptides correspond to the carboxyl end of the α chain, as reported by Prahl *et al.* (1971).

Peptides TPα1 and TPα1a. These peptides were obtained after peptic digestion of the precipitate. The sequence of peptide TPα1 was determined by the dansyl-Edman procedure and is: Ser-Leu-Cys-Ser-Thr-Glx-Pro-Asx-Gly-Asx. This peptide includes TPα1a: Cys-Ser-Thr-Glx-Pro-Asx-Gly-Asx which was produced by a further peptic split of the first two residues Ser-Leu, at the amino-terminal end. This peptide has the same composition and mobility at pH 6.5 as one which was bridged to the carboxy-terminal peptide of the κ chain, isolated from a peptic–tryptic diagonal map of this protein. This result is in agreement with previous

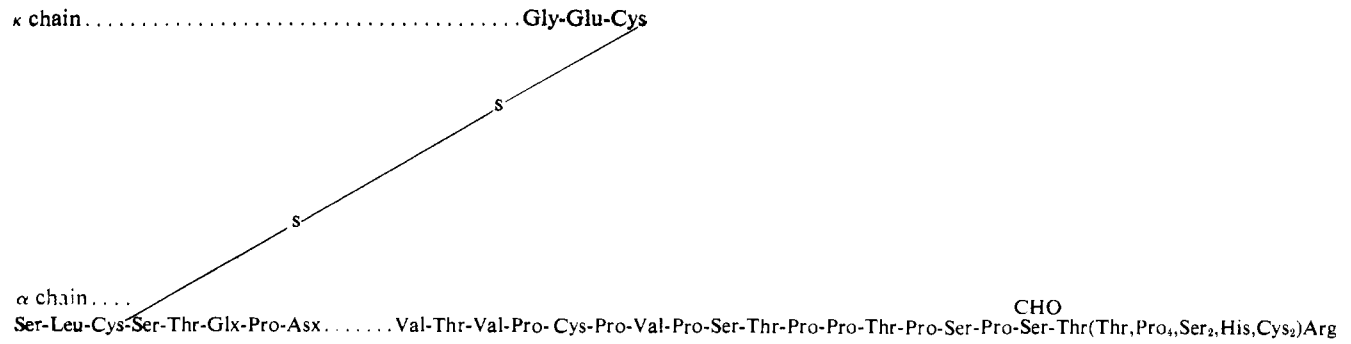

FIGURE 2: Sequences around the interchain disulfide bridges of protein Pat (γA1).

studies on another γA1-immunoglobulin (Mihaesco *et al.*, 1971). A peptide having similar composition, mobility, and staining characteristics was isolated by diagonal electrophoresis from a γA2, Am2 (−) protein (Rou) (Mihaesco *et al.*, 1971) and its partial sequence, as given by the dansyl-Edman procedure was identical with peptide TPα1a (Table IV).

Discussion

In the present study, a γA1 myeloma was partially reduced and carboxymethylated with radioactive iodoacetic acid in conditions which have been found in immunoglobulins G and M, to label interchain disulfide bonds (Miller and Metzger, 1965; Pink and Milstein, 1967; Frangione *et al.*, 1969b). The heavy chain was subjected to proteolytic digestion and the labeled peptides were purified and sequenced. Of the six major peptides thus characterized only three have been clearly localized in the molecule. One, (TPα6) was recognized as being similar to the previously characterized carboxy-terminal end of the α chain and was found both with and without the terminal tyrosine, probably due to either peptic cleavage of the Cys–Tyr bond or possibly *in vivo* carboxypeptidase activity (Prahl *et al.*, 1971). Another (TPα2) had a composition that showed the high content of proline and cysteine which is typical of the hinge region of other immunoglobulin classes and is therefore considered to contain the interheavy-chain bridges (Frangione *et al.*, 1969a). Its sequence was established up to the 18th amino acid. According to its mobility and amino acid composition, this peptide probably contains three carboxymethylcysteines. However, it was not possible to ascertain the exact number because of the low recovery of CM-cysteine in the amino acid analyzer and the presence in the peptide of histidine (partially charged at pH 6.5) and carbohydrate. There remains some question if all these cysteine residues are involved in the heavy–heavy bridge since preliminary studies by diagonal electrophoresis of another γA1 myeloma protein (OSO) revealed peptide TPα3 in the same region of the map. A third peptide is the one containing the heavy–light disulfide bridge (TPα1). It was also obtained in two forms, one resulting from peptic cleavage of the first two residues Ser-Leu from the amino-terminal end of the other. It is of interest that the sequence of this peptide from the γA1 proteins is exactly the same as that of the rare γA2 Am2 (−) genetic variant (Table IV) thus suggesting a close evolutionary relationship between them. In contrast, Mihaesco *et al.* (1971) did not find the characteristic peptide in the diagonal map of a γA2k Am2 (+) myeloma protein which lacks the heavy–light disulfide bond. Comparison of the sequence of the heavy–light peptide with the corresponding peptides of other immunoglobulins (Table IV), shows some degree of homology with the corresponding peptides from the γ2, 3, and 4 subclasses and suggests that it is in the middle of the Fd region rather than near the hinge. The sequences around the heavy–light and heavy–heavy disulfide bridges are shown in Figure 2.

The function of the remaining carboxymethylcysteine peptides is still unknown. They could represent labile intrachain bonds, additional interchain bridges or possibly a disulfide bridge to the recently described J chain which also seems to be bound to the α chains.

Added in Proof

The hinge region obtained from a γA2 protein is homologous to that present in γA1 but has a gap of about nine residues and lacks carbohydrate (Frangione *et al.*, 1971).

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Hydrogenation of Reduced Aldol Condensation Product from Elastin*

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ABSTRACT: The catalytic hydrogenation of the reduced aldol condensation product isolated from reduced bovine elastin yields several products. Isolation of these products was achieved by ion-exchange chromatography. The structures of the most abundant products were determined by mass spectrometry. Results obtained were consistent with the presence of an allylic alcohol group in the previously isolated reduced aldol condensation product.

Lent *et al.* (1969) have reported the presence of a reduced aldol condensation product (ACP;[1] I) in alkaline hydrolysates of elastin previously reduced with $NaBH_4$. Available data suggest that this compound arises in elastin from the condensation of two residues of α-aminoadipic acid δ-semialdehyde, the latter resulting from the enzymatic deamination of lysyl residues previously incorporated into the polypeptide chains of elastin (Salcedo *et al.*, 1969).

$$\underset{\text{I}}{HOOCCH(NH_2)(CH_2)_2C(CH_2OH)=CH(CH_2)_3CH(NH_2)COOH}$$

Previous evidence presented for the structure of the reduced ACP included the mass spectrum of its ethyl ester derivative as well as studies on the periodate–permanganate oxidation of the compound (Lent *et al.*, 1969). However, the position of the double bond has not been clearly established.

This communication presents additional studies on the catalytic hydrogenation of the reduced ACP. The data support the presence of an allylic alcohol in the structure proposed.

Experimental Section

Isolation of the reduced ACP from elastin was described previously (Lent *et al.*, 1969). The reduced ACP used in these experiments was isolated from 200 mg of bovine elastin, which had been previously reduced with $NaBH_4$. The elution pattern of the reduced ACP from the Technicon amino acid analyzer empolying the gradient of Burns *et al.* (1965) is given in Figure 1.

Hydrogenation was performed in a Parr apparatus as follows. Approximately 1.0 μmole of reduced ACP from elastin was dissolved in 0.6 ml of 0.1 N HCl. To this was added 5.0 ml of H_2O and 15 mg of palladium black (Fisher Scientific Co., Lot 752241). Hydrogenation was then carried out in 2 atm of H_2 gas for 18 hr at room temperature. The reaction mixture was filtered and evaporated to dryness. The residue was dissolved in 1.0 ml of 0.01 N HCl.

Separation of the reaction products from the hydrogenation experiment was achieved by use of the Technicon amino acid analyzer employing the gradient described by Burns *et al.* (1965). A typical chromatogram of the reaction mixture is also shown in Figure 1. All ninhydrin-positive peaks were arbitrarily given a designated letter, A through E. In the several experiments performed, compound A and compound E were the major ninhydrin-positive fractions which appeared on the amino acid analyzer after hydrogenation. Together they accounted for 75–80% of the total ninhydrin reactivity recovered from the reaction mixture; compound A varied from 10 to 25% of the total ninhydrin, while compound E varied from 50 to 75%. Isolation of the individual compounds was accomplished by employing a stream-splitting device. Pooled fractions were desalted on a Dowex-50 column employing 0.25 M NH_4OH as the elution buffer.

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[1] Abbreviation used is: ACP, dehydrated aldol condensation product of two residues of α-aminoadipic acid δ-semialdehyde.