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7. Bio-Sil HA, minus 325 mesh, obtained from Bio-Rad Laboratories, Richmond, Calif.
8. The hexane used in these investigations was purified to the equivalent of spectral grade by percolation of reagent-grade hexane through silica gel and distillation. The ethyl ether was distilled and stored over sodium. The methanol was reagent-grade that was distilled before use.
9. Melting points are corrected; boiling points are uncorrected.
10. R_F values obtained by developing chromatograms with the ethanol-ammonium hydroxide system (95:5) were frequently not reproducible. This difficulty was overcome by increasing the ammonia in the developing solvent.
11. The vapor phase chromatography was carried out on an F & M instrument equipped with a Model 1609 flame-ionization attachment by using a stainless steel column (3.7 m by 0.31 cm diameter) packed with 10-percent diethyleneglycol succinate on acid-washed Chromosorb W; column temperature, 95°C; nitrogen flow rate, 20 ml/min.
12. Commercially available valeric acids almost always contain varying percentages of the structural isomers as contaminants, but the pure isomers were obtained by slow distillation through a long, straight-tube column. The methyl esters were prepared by refluxing the respective acids for 4 to 5 hours with methanol containing 5 percent of gaseous hydrogen chloride, boiling off the methanol, washing an ether solution of the ester with cold water, and fractionally distilling the product at atmospheric pressure through a straight-tube assembly.

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Protein Conformation in Solution: Cross-Linking of Lysozyme

Abstract. *The cross-linking of lysozyme by reaction with phenol-2,4-disulfonyl chloride has been effected. The cross-linked protein retained enzymatic activity, has approximately the same molecular weight as native lysozyme, and has essentially the same conformation as native lysozyme as judged by optical rotatory dispersion analysis. The positions of sulfonylation were assigned by a standard degradation sequence; the presence of sulfonamide bonds was confirmed by infrared spectroscopy. Cross-links may thus be introduced without incurring major structural changes in the protein, and certain intramolecular distances that are allowed in the active enzyme may be deduced.*

The primary structure of many proteins has now been worked out (*1*). A few of these have been studied by means of x-ray crystallographic techniques. The position of each atom heavier than hydrogen can be deduced from the x-ray data, and this has been done in the case of myoglobin (*2*). In other proteins the position of the backbone polypeptide has been determined, even though the exact positions of the side chains of the polypeptide chain (or chains) remain largely unknown.

It is important to determine whether a protein has the same three-dimensional structure in solution as it does in the crystalline form. Lysozyme is a protein that is amenable to such a comparison. Egg-white lysozyme has been studied in great detail; its primary structure has been determined (*3*), and a partial description of the three-dimensional structure has been set forth (*4*). Many physical methods have been utilized to study various aspects of the structure of lysozyme; frequently the information determined by physical methods can indicate the nature of various combinations of certain functional groups in juxtaposition, that is, hydrogen bonding, helical structure, but these methods cannot, in general, determine the position of functional groups in the amino acid sequence nor necessarily to structures proposed on the basis of x-ray diffraction studies.

The cross-linking of globular proteins with reagents of fixed geometry appears to be a tool of potential value in determining certain spacings within a protein molecule; studies with various reagents have been reported, although few assignments of cross-linking positions have been made (*5*).

We now report a method for assigning certain allowed distances within the lysozyme molecule and to establish minimum spacings for other groups. Lysozyme was subjected to cross-linking by phenol-2,4-disulfonyl chloride under essentially isoionic conditions. The isoionic conditions were maintained during the time of the cross-linking by suspending both the cation-exchange resin Dowex-50W-X12 (H form) and the anion exchange resin Dowex-1-X12 (OH form) in a solution of lysozyme (1 mg/ml). Lysozyme was not significantly adsorbed nor denatured by this treatment. Lysozyme was recovered in good yield and with complete retention of enzymatic activity, in control experiments. S^{35}-Labeled phenol-2,4-disulfonyl chloride dissolved in acetone was added slowly (approximately over an 8-hour period) to the gently stirred slurry of the resin particles in the lysozyme solution, which was essentially neutral throughout the process (approximately *p*H 7), hydrolyzed excess reagent was removed from the solution quantitatively during the run as demonstrated by the very low radioactivity in

Table 1. Amino acid composition of lysozyme peptides and assignment to primary structure. The letter T (last column) means tryptide; the subscript designates the position in the primary sequence (*3*). The abbreviations of the amino acid residues are: Lys, lysine; Arg, arginine; Cmc, carboxymethyl cysteine; Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; and Tyr, tyrosine.

Peptide *	Presence of amino acids †																Assignment ‡
	Lys	Arg	Cmc	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met§	Ile	Leu	Phe	Tyr	
A	+	+	+	+	+	+	+	○	+	+	+	○	+	+			$T_3T_4T_{11}T_{12}T_{13}$
A_{11}	+		○				+			+		○		+			T_3
A_{12}		+															T_4
B	+	+	+	+	○	+	+	+	+	+	+	○	+	+	○	+	$T_6T_7T_{11}T_{12}T_{13}$
B_{22}	+		+	+		○			+	+	○			+			T_6
B_{24}		○		+	+	○	○			+				●	+	○	T_7
B_{31}	+		+	○	+	+		○		+	+		+	+			T_{11}
C	+	+	+	+	+	+	+		+	+	+	○	+	+	○	+	$T_6T_7T_{15}T_{16}$
C_{21}	+		○	○		○			+	+	○			+			T_6
C_{22}		○		+	+	+	○			○					+	○	T_7
C_{51}	+	+	+	○	+		+		○	+	+		+				$T_{15}T_{16}$
C_{52}	+		○														T_{15}

* The letters A, B, and C represent radioactive peptides derived by tryptic digestion of one of the fractions of cross-linked lysozyme. Each peptide (A, B. . .) was treated with hydrobromic acid (HBr) to give A_1, A_2, A_3. . . . Each of these was subjected to a second tryptic digestion: $A_1 \rightarrow A_{11}$ and A_{12}, and others, according to the procedure described (*6*). † Meaning of symbols: +, found and predicted from the Canfield sequence; ○, not found but predicted; ●, found but not predicted. ‡ The compositions observed by thin-layer chromatography were matched to the reported tryptide. Compositions reported by Canfield (*3*). § Methionine apparently modified to sulfoxide or sulfone (or both) by released bromine in HBr cleavage. It was not positively identified.

the low-molecular-weight region of a gel-filtration profile. The ion-exchange beads were removed by filtration, and the protein was recovered by lyophilization. The protein could be resolved into four components both by Tiselius electrophoresis and by chromatography on carboxymethyl-Sephadex with a salt gradient (the activity coefficient was 0.5 initially, and 2.0 finally; approximately linear; a *p*H 8 phosphate buffer was used throughout). Three of these fractions were radioactive and contained approximately 1,2 and 1 residue of phenol-2,4-disulfonyl chloride per molecule of lysozyme. On the basis of mobility, point of elution, and specific radioactivity it would appear that the slowest-moving peak electrophoretically, which corresponds to the first peak eluted from the carboxymethyl-Sephadex, is a dimer. The dimer (less than 10 percent of reaction mixture) contains two cross-linking residues, one of which at least is involved in an intermolecular linkage. The fourth peak was established to be unreacted lysozyme. The fractions had 25, 80, 74, and 98 percent of the enzymatic activity of the control. The presence of large amounts of dimer was ruled out by analysis of the data obtained from a Spinco Model E ultracentrifuge. The optical rotatory dispersion (ORD) spectra of the lysozyme and the cross-linked fractions gave identical rotations above 320 mμ; that there was some difference below 320 mμ may be indicative of unknown structural change.

In order to locate the positions at which the cross-linking reagent had reacted, the appropriate fractions were subjected to the sequence of reactions (*6*) that provides for the generation of sets of tryptides (peptides from a digestion by trypsin) and their separation. The determination of the amino acid composition of each fragment allowed an unambiguous assignment of the position of cross-linking to a particular residue in the sequence (Table 1). Amino acids were reported if the spot on the thin-layer chromatogram was of comparable intensity to the other spots and at the exact position of the two controls. Assignment of a particular composition to a given tryptide sequence was made on the basis that, even though the analytical data were incomplete, there was only one Canfield tryptide that contained the amino acids identified in our work, and that did not also contain several amino acids not found here. Thus the data in Table 1 indicate that three cross-links were introduced—only one in any given molecule—and these are between lysines at positions 33 and 96, 33 and 97, and between 33 and 116 [numbers in primary sequence according to Canfield (*3*)]. Most, if not all, of the ε-amino residues and perhaps the α-amino group have been sulfonylated; cross-linking can follow in some cases, and in the others none is seen to occur. Sulfonylation without cross-linking can arise from hydrolysis of one of the $-SO_2Cl$ groups before, or after, sulfonylation by the other. In addition to showing that particular amino acid residues are involved in the cross-linking, the infrared spectra of the appropriate fractions showed new absorptions characterized as arising from sulfonamide bonds (*7*).

We conclude that (i) the cross-linking has resulted in the introduction of new covalent bridges between pairs of ε-amino groups, (ii) most of the amino groups are "available" for sulfonylation even if not for cross-linking, and (iii) the cross-linking has not resulted in any substantial change in enzymatic activity. The ORD spectrum shows no measurable difference when compared with the control above 320 mμ and some between 220 and 320 mμ; taken with the enzyme-activity data it must mean that a degree of conformational integrity is retained. However, more detailed examination (*8*) reveals that the cross-links can only be formed by rearranging the side chains of lysine. This is not surprising since it would have seemed unlikely, a priori, that any reagent could match exactly the spacing requirements for any potential cross-link. Thus, a certain degree of rearranging from the crystalline structure must be expected. If the rearrangement required is too extensive (*9*), the described cross-linking process per se may be suspected of causing changes in the structure. On the other hand, a consistent pattern of cross-linking of pairs of functional groups of reagents of different chemical properties or different geometry (or both) may be presumptive evidence that the side chains of the amino acid residues are free to move when the protein is in solution.

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Durum-Type Wheat with High Bread-Making Quality

Abstract. *A durum-type wheat (2n = 4x = 28) with high bread-making quality was produced by crossing a durum-wheat variety with a common bread-wheat variety (2n = 6x = 42), backcrossing to the bread-wheat variety for three generations, and then selecting for 28-chromosome plants. The high quality is tentatively attributed to a translocation involving one of the D-genome chromosomes.*

The fact that the hexaploid wheat of agriculture (*Triticum aestivum* L. em. Thell.), genomically AABBDD, has been produced by the addition of the chromosomes of *Aegilops squarrosa* L., genomically DD, to the tetraploid emmer wheat (*Triticum dicoccoides* Korn. in litt. in Schweinf.), genomically AABB (*1*), is generally accepted. The varieties of present-day durum wheat, *T. durum* Desf., originated from emmer wheat. These two main classes of cultivated wheat differ in a number of characteristics, primarily in yield in favor of the durum wheat (*2*) and in bread-making quality in favor of the hexaploid wheat. This better bread-making quality of the hexaploid wheat has been attributed to the influence of the D-genome chromosomes, although there are conflicting reports regarding the chromosomes that carry the genes for quality (*3*). The desirability of producing a durum wheat with satis-