As shown in Fig. 1, more acetaldehyde was formed in tissue extract incubated at 25 °C than in ascorbic acid solution. An explanation for this may be that tissue extracts usually contain activators like Cu^{2+} and Fe^{3+} which catalyse the autoxidation of ascorbic acid, especially at low temperatures.[7] At physiological pH, however, the formation of acetaldehyde is much less since the reaction becomes much slower as the pH rises over 5.[1] In addition, ascorbic acid-rich tissues also contain a relatively high amount of SH groups,[8,6,5] which inhibit the non-enzymic oxidation *in vitro*.[1] It can be assumed, therefore, that aliphatic alcohols cannot be oxidized to aldehydes by ascorbic acid *in vivo* at such a speed that the products would produce harmful effects.

Experimental. Bovine liver, kidney, heart and blood were separately homogenized in 0.6 N perchloric acid (PCA) (35 g tissue/100 ml) and the homogenates filtered. Before use, the pH of the acidic filtrate was adjusted to 4.0 by adding 1.25 ml of 5 M KOH and 1 M citrate buffer (pH 4.0) to 10 ml of the filtrate. The samples were incubated for 60 min in a thermostated water bath in the presence of different aliphatic alcohols. After the incubation thiourea was added to prevent alcohol oxidation during the subsequent analysis of the aldehydes. Thiourea was added to the control samples before incubation. In two experiments 0.1 M citrate buffer, pH 4.0, containing different amounts of ascorbic acid was used instead of tissue extract. The ascorbic acid content was estimated by the method described by Roe.[9] The alcohols and aldehydes except formaldehyde were estimated on a Perkin-Elmer F 40 head-space gas liquid chromatograph, as reported previously.[10] Formaldehyde was estimated spectrophotometrically by the violet color which develops on heating with chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) in the presence of strong sulfuric acid. The absorbance was measured at 570 nm.[11]

The analytical procedure was standardized with samples of diluted formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, valeraldehyde, isovaleraldehyde, methanol, ethanol, propanol, butanol, isobutanol, pentanol, and isopentanol of analytical grade. Standard and unknown samples were determined by identical procedures.

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Correction of the Amino Acid Sequence of Soybean Leghemoglobin *a*

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A comparison of the sequence of soybean leghemoglobin *a* (Lb*a*) [1,2] with the sequence of soybean leghemoglobin *c* (*to be published*) and kidney bean leghemoglobin *a* [3] revealed a genetically inexplicable difference at the site 50 – 54 in the leghemoglobin *a* chain. The order of these amino acids in both soybean leghemoglobin *c* and kidney bean leghemoglobin *a* is *Gly-Val-Asp-Pro-Thr*, whereas *Pro-Thr-Asp-Gly-Val* was proposed for leghemoglobin *a* from soybean. This

Table 1. Amino acid compositions of substilopeptidase A peptides from the tryptic peptide *a*T14.

Amino acid	S1	S2	S3	S4	S5
Aspartic acid	1.13	1.02		2.06	0.89
Threonine				0.97	
Serine			0.94		
Proline				1.90	
Glycine				0.53	
Alanine		1.13			1.11
Valine				1.07	
Leucine	0.94	0.86			
Phenylalanine	0.92		1.06		
Lysine				1.02	
E_{asp}	0.52	neutr.	neutr.	neutr.	neutr.

prompted us to re-investigate the sequence of the tryptic peptide *a*T14.

Materials and methods. The methods used were mainly those described previously.[2] Peptide *a*T14 was isolated from the tryptic hydrolysate of apoLb*a* using high voltage electrophoresis at pH 6.5, pH 1.9, and paper chromatography. Peptide *a*T14 was digested with subtilopeptidase A (Sigma Chemical Company, St. Louis, USA), and the peptides were isolated as described previously. The amino acid composition of the peptides was determined on a modified Beckman/Spinco 120 amino acid analyzer. The sequence of the partial peptides was determined by the dansyl-Edman method.[4] The dansyl amino acids were identified by high voltage electrophoresis on silica gel plates.[5] or by miniature thin layer chromatography on polyamide sheets (3 × 3 cm).[6]

Results and discussion The amino acid contents of the subtilopeptidase A peptides (denoted by S) are presented in Table 1. The low glycine content of peptide S4 indicates that it is a mixture of two peptides. The only difference between the peptides is at the amino end, at which the *N*-terminal glycine is missing from the smaller one (Fig. 1). This minor difference makes it impossible to separate the peptides by conventional means.

In the previous determination of the sequence of *a*T14, the subtilopeptidase A peptides were obtained in very low yield and, apparently contaminated by impurities, which led to an incorrect deduction of the sequence. The chymotryptic [7] and thermolytic [8] hydrolysates of apoLb*a* also yield peptides covering this portion of the Lb*a* chain. However, these peptides, *a*C18 and *a*Th10, respectively, were investigated only to elucidate the bridges between the tryptic peptides and were therefore not completely sequenced. Consequently the misinterpretation of the data was not observed. The correct sequence of peptide *a*T14 is given in Fig. 1.

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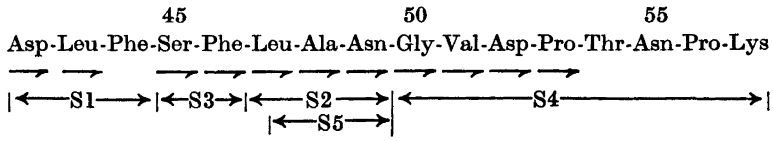

Fig. 1. The amino acid sequence of the tryptic peptide *a*T14 (residues 42 – 57 in the Lb*a* chain). The arrows show the amino acids identified by the dansyl-Edman method. The subtilopeptidase A peptides are shown below the sequence.