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ENANTIOMER LABELLING, A METHOD FOR THE QUANTITATIVE ANALYSIS OF AMINO ACIDS

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SUMMARY

Enantiomer labelling, a method for the quantitative analysis of optically active natural compounds by gas chromatography, involves the use of the unnatural enantiomer as an internal standard. With Chirasil-Val, a chiral stationary phase that is thermally stable up to up to 240°, the enantiomers of amino acids and a variety of other compounds can be separated and quantitated. Incomplete recovery from the sample, incomplete derivatization, hydrolysis and thermal decomposition of the derivative and shifting response factors can be compensated for by adding the unnatural enantiomer. The accuracy of amino acid analysis by enantiomer labelling is equal or superior to that of hitherto known methods. The procedure affords a complete analysis of peptides with respect to both amino acid composition and the optical purity of each amino acid.

INTRODUCTION

Quantitative amino acid determination is a basic method for the analysis of synthetic and natural peptides and proteins and serves as a diagnostic tool in clinical chemistry. The most widely accepted procedure, based on the work of Spackman *et al.*[1] utilizes ion-exchange chromatography, on-line reaction of the eluate with ninhydrin and monitoring of the absorbance of the resulting chromophore. Under optimal conditions, the method is capable of a precision of approximately ± 5% depending on the amino acid (for proline up to 10%), with a sensitivity of 10–100 nmole depending on the instrument. Disadvantages of the method are the necessity for a dedicated and expensive instrument, the long time of analysis (3–4 h per cycle) and a sensitivity that is unsufficient for many applications.

A more recent method involving the separation of dansylamino acids by high-performance liquid chromatography on silica gel and fluorimetric detection[2] should reduce the time of analysis and increase the sensitivity considerably. The quantitative reproducibility of the method has not yet been fully assessed.

Gas chromatography of amino acids is less widespread (for a review, see ref. 3). Gehrke and co-workers[4–6] optimized the derivatization and gas chromatographic conditions so as to permit the quantitative analysis of all amino acids. The time

required for one analysis is less than 1 h and no specialized equipment is needed. The sensitivity is at least two orders of magnitude higher than in the ion-exchange procedure. However, the gas chromatographic method also possesses inherent weaknesses. The rate of derivatization differs from one amino acid to another, thus requiring strict reproduction of the reaction conditions for all samples. Considering the number of steps involved and reagents used, this requirement is difficult to fulfil.

Some amino acids need special treatment for complete derivatization and some derivatives are extremely sensitive to moisture. The most volatile amino acid derivatives may be lost during concentration of the sample. In addition, for each change of the chromatographic conditions, *i.e.*, gas flow-rates, temperature programme, type of detector, etc., the response factors have to be determined anew.

These weaknesses could be overcome if each amino acid could be co-derivatized and co-chromatographed together with an internal standard with identical chemical properties, *i.e.*, its optical antipode. A similar approach using diastereomers as internal standards has been proposed earlier[7]. In our view, this method is less suitable, as diastereomers are chemically non-identical and the rate of reaction between two chiral reactants is not independent of their configuration. The use of enantiomers as internal standards has also been considered[7], but with no substantiating experimental proof.

The use of the optical antipode as an internal standard has distinct advantages. The standard is added before any manipulations are executed in order to ensure that both standard and sample are subjected to identical treatment. Errors due to incomplete recovery and derivatization, decomposition, losses and differing detector responses are thus completely compensated. No external standard is required for the calculation of the aliquot injected on to the column.

A prerequisite to a method using the optical antipode as an internal standard is a chromatographic system that is capable of separating the enantiomers. Chiral stationary phases for this purpose have been described in the past, but none of them afforded the separation of all protein amino acids. The method of enantiomer labelling has become feasible since the introduction of Chirasil-Val*, a chiral polysiloxane stationary phase of high thermal stability[8]. In our experience, satisfactory separations of all amino acids can only be achieved with capillaries, and in this work glass capillaries were used throughout.

EXPERIMENTAL

Preparation of samples

An 80-nmole amount of peptide is weighed into a heavy-walled hydrolysis tube, 1 ml of 6 *N* hydrochloric acid and 0.1 ml of mercaptoethane are added and the sample is hydrolysed *in vacuo*. After 24 h at 110°, the tube is opened and the sample is transferred quantitatively into a round-bottomed flask and evaporated to dryness. The residue is dissolved in a weighed amount of water and two accurately weighed aliquots are taken. The first aliquot is evaporated to dryness under a gentle stream of nitrogen with moderate heating. For removal of residual water, the sample is taken

* Chirasil-Val is commercially available from Applied Science Labs., State College. Pa., U.S.A.

up in acetone–benzene and concentrated again twice. The sample is derivatized and chromatographed as described below.

A corresponding, accurately weighed amount of standard solution is added to the second aliquot, and the sample treated as described above.

Standard solution

A mixture of 20 μmole of each D-amino acid (supplied by Sigma, St. Louis, Mo., U.S.A., and Serva, Heidelberg, G.F.R.) is placed in an accurately weighed volumetric flask. The amino acids are dissolved in 80 ml of water containing a drop of hydrochloric acid and 0.01% azide. The solution is diluted to 100 ml, accurately weighed and the amount of each amino acid contained in 1 g of solution is calculated.

Derivatization

Esterification. A 1-ml volume of 2 *N* hydrochloric acid in isopropanol is added to the dry sample. Air is displaced with nitrogen and 1 ml of mercaptoethane is added to prevent oxidative decomposition of tryptophan and to convert cystine into cysteine. The reaction mixture is heated at 110° for 1 h. Solvent and excess of reagents are removed with a gentle stream of nitrogen.

Acylation. A 250-μl volume of ethyl acetate and 50 μl of pentafluoropropionic anhydride are added and the reaction mixture is heated at 110° for 10 min. If arginine is to be determined, the temperature must be increased to 150°. Solvent and excess of reagent are evaporated with a gentle stream of nitrogen. The residue is dissolved in a known volume of methylene chloride and an aliquot representing 40 pmole is injected for gas chromatography.

N^{im}-Ethoxycarbonylhistidine[9]. A 100-μl volume of benzene and 2 μl of diethyl pyrocarbonate are added to the dry residue of N,O,S-acyl amino acid isopropyl ester, most conveniently the solution remaining from the gas chromatographic analysis of all other amino acids. Derivatization is effected in a heavy-walled screw-capped vial, *e.g.*, a Reacti-vial reinforced above the septum with a small steel disc, at 150° for 20 min. The solvent is evaporated and the mixture is re-dissolved in an appropriate volume of methylene chloride. Gas chromatography is performed isothermally at 175°.

Chromatography

For the gas chromatographic analysis, a Carlo Erba Model 2101 gas chromatograph equipped with glass capillaries (20 m × 0.3 mm) coated with Chirasil-Val (film thickness *ca.* 0.1 μm) is used with hydrogen as the carrier gas at an inlet pressure of approximately 0.35 bar. The column is programmed as follows: 5 min isothermal at 87°, 4°/min to 200°, then maintained at 200°. The peak areas are computed with an electronic integrator (Spectra Physics System I).

RESULTS AND DISCUSSION

Enantiomer labelling as a method for the quantitative determination of optically active compounds is based on the valid assumption that optical antipodes exhibit identical chemical and physical properties in a non-chiral environment. Stereospecificity is a prevalent phenomenon of biological systems; hence this analytical method can be recommended for the analysis of natural compounds and especially

for the compound class which probably represents the prime principle of this specificity, namely the amino acids.

A prerequisite for the general applicability of enantiomer labelling is a chromatographic system that is capable of separating all amino acid enantiomers. This can be achieved by using capillaries coated with Chirasil-Val; Fig. 1 shows a typical chromatogram of the separation of all enantiomers of the 18 protein amino acids. The analysis of peptides and proteins of any origin by enantiomer labelling combines the determination of two features of equal importance for a peptide, namely the absolute or relative molar proportions of the constituent amino acids and, necessarily, their respective optical purities. As the calculation of the amount of a natural L-amino acid present in a sample is based on the known amount of unnatural D-amino acid enantiomer added as a standard, the respective enantiomeric impurities have to be taken into account. Therefore, the amounts of the respective antipodes in both the standard and the sample are determined first.

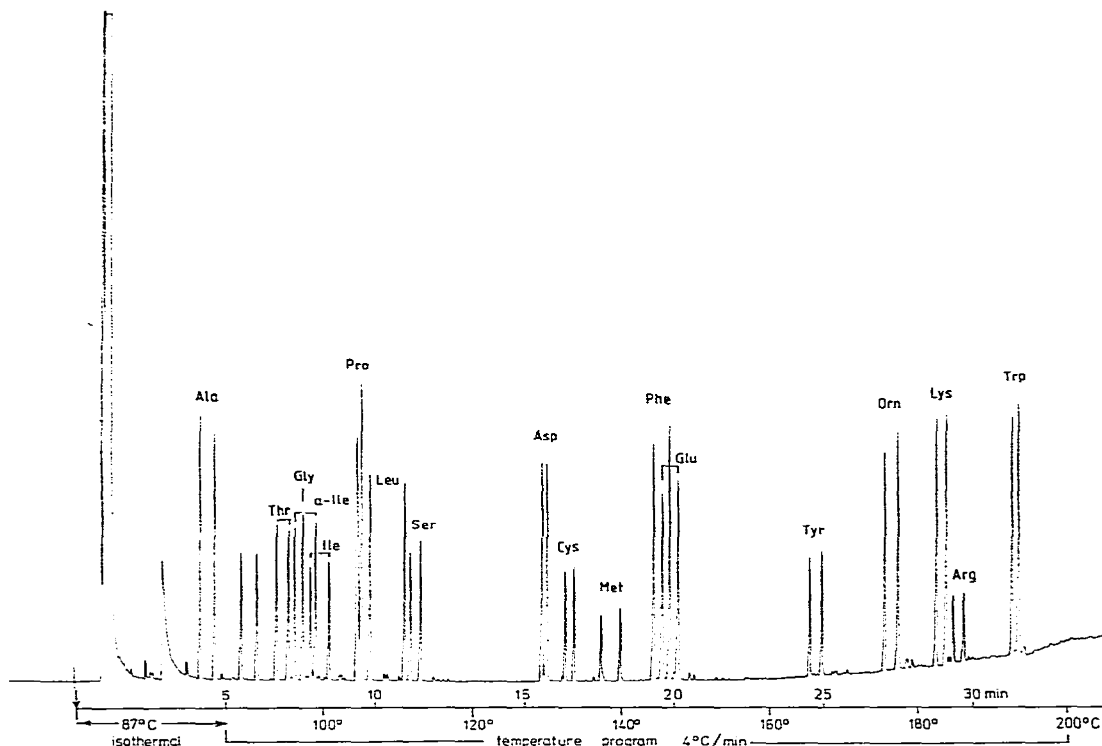

Fig. 1. Gas chromatographic separation of a racemic mixture of 19 protein amino acids on a 20-m capillary of Chirasil-Val.

The D-amino acids used as internal standards showing the correct elemental composition were tested for their optical purity. The proportions of L-enantiomers found in the standard D-amino acids are summarized in Table I.

Similarly, the amount of D-amino acid in the sample is determined, thus roviding the first important analytical data, namely the extent of racemization of

TABLE I

PERCENTAGES OF L-AMINO ACIDS FOUND IN THE D-AMINO ACIDS USED AS ENANTIOMER LABELS

Mean of four determinations. C_D represents the ratio of L-enantiomer to D-enantiomer and serves as correction factor for calculation of the amount of L-amino acid in a sample (see below).

D-Amino acid	*L-enantiomer (%)*	$C_D \cdot 10^2$
Alanine	0.08	0.08
Valine	1.47	1.49
Threonine	0.59*	(0.60)
Isoleucine	2.45*	(2.6)
Proline	1.18	1.19
Leucine	3.22	3.33
Serine	1.01	1.02
Aspartic acid	0.52	0.52
Cysteine	0.17	0.17
Methionine	2.36	2.41
Phenylalanine	0.91	0.92
Glutamic acid	2.09	2.13
Tyrosine	2.10	2.15
Lysine	2.04	2.08
Tryptophan	5.11	5.38

* Racemization to the *allo*-L-amino acids, which does not contribute to the areas of the corresponding *threo*-L-amino acids present in natural samples.

each amino acid. Simultaneously, the total concentration of the amino acids present in the sample is calculated from this determination; a comparable amount of enantiomeric standard is added to a second aliquot. Finally, this second aliquot is derivatized, chromatographed and the peak areas integrated electronically.

Optical impurities in the natural L-amino acids or in the enantiomeric labels do not preclude a quantitative determination but must be considered in the calculation. The amount of a particular amino acid (X_a) present in the sample is calculated from the ratio of the areas of the L-enantiomer (A_S) and D-label (A_T) multiplied by the amount of label added (m_D):

$$X_a = m_D \cdot \frac{A_S}{A_T} \tag{1}$$

assuming linearity of detector response *versus* concentration of both enantiomers. However, this applies only to the ideal case when both enantiomers are free from racemate. Racemization of the natural L-amino acid contributes to the area of the D-standard and *vice versa*.

Therefore, correction factors as given in Table I for the enantiomeric labels and also for the amino acids to be quantitated must be considered, the latter being acquired from ascertainment of the extent of racemization in the sample.

For the calculation of the "true" area, A_S, representing the amount of amino acid in the injected aliquot, the peak area of the L-enantiomer, A_L, must be corrected. The first correction is for the fraction of racemized, added "D"-standard, which contributes to the total peak area of L-enantiomer, A_L, and which is of size $A_D C_D$

(C_D = ratio of L-enantiomer to D-enantiomer in the standard mixture). Thus, the first approximation to the true area, A_s, is

$$A_{L,1} = A_L - A_D C_D \quad .$$

The approximated area $A_{L,1}$ is now used for calculation of the fraction of the amino acid sample racemized to the D-enantiomer, which has to be added for a second approximation, and which is of size $A_{L,1}C_L = (A_L - A_D C_D)C_L$ (C_L = ratio of D-enantiomer to L-enantiomer in the sample). The better approxomated area is now

$$A_{L,2} = A_L - A_D C_D + (A_L - A_D C_D)C_L$$

The last term, $(A_L - A_D C_D)C_L$, on the other hand, is also the fraction of the area, A_D, which actually does not arise from the added D-standard. The area A_D used for calculation of the second term of the series is therefore too large by the fraction represented by $(A_L - A_D C_D)C_L$, and consequently the area subtracted is too large by the fraction $(A_L - A_D C_D)C_L C_D$. The third approximation is then

$$A_{L,3} = A_L - A_D C_D + (A_L - A_D C_D)C_L + (A_L - A_D C_D)C_L C_D \quad .$$

If this process is repeated *ad infinitum*, the "true" area, A_s is achieved:

$$A_{L,n} = A_s = A_L - A_D C_D + (A_L - A_D C_D)C_L + (A_L - A_D C_D)C_L C_D + + (A_L - A_D C_D)C_L^2 C_D \ldots \quad (2)$$

TABLE II

QUANTITATIVE DETERMINATION OF AMINO ACID CONCENTRATION (C_f) IN A SYNTHETIC MIXTURE, OBTAINED THROUGH ENANTIOMER LABELLING, FOR VARIOUS AMOUNTS OF A D-AMINO ACID MIXTURE ADDED AS INTERNAL STANDARD

R = ratio of D-standard to L-amino acid in the mixture. σ = Standard deviation (n = 4). Δ = Absolute errors (as percentage of the nominal amount of amino acid in the sample).

Amino acid	*Nominal concentration (μmole/ml)*	*R = 5.7*			*R = 4*			*R = 2.3*		
		C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)
Alanine	3.044	2.980	− 2.1	1.1	2.959	−2.8	0.8	3.050	+0.2	0.9
Valine	2.342	2.377	+ 1.5	4.6	2.389	+2.1	1.0	2.419	+3.3	1.3
Threonine	2.582	2.488	− 3.6	0.9	2.422	−6.2	0.8	2.580	+0.1	2.4
Isoleucine	2.332	2.366	+ 1.5	1.0	2.257	−3.2	1.1	2.419	+3.7	1.8
Proline	2.717	2.922	+ 7.5	2.0	2.766	+1.8	3.4	2.875	+5.8	0.8
Leucine	2.156	2.063	− 4.3	1.2	2.111	−2.1	0.4	2.119	−1.7	1.7
Serine	2.993	2.722	− 9.1	0.6	2.763	−7.7	1.5	2.816	−5.9	3.6
Aspartic acid	2.385	2.348	− 1.6	1.5	2.311	−3.1	0.9	2.363	−0.9	0.5
Cysteine	2.262	n.d.			n.d.			n.d.		
Methionine	2.060	1.995	− 1.6	4.8	2.041	−0.9	2.3	2.086	+1.3	1.1
Phenylalanine	1.897	1.713	− 9.7	0.2	1.815	−4.3	3.2	1.916	+1.0	1.8
Glutamic acid	2.324	2.196	− 5.5	0.7	2.270	−2.3	0.6	2.207	−5.0	1.9
Tyrosine	1.593	1.476	− 7.3	2.3	1.544	−3.1	10.7	1.534	−3.7	1.8
Lysine	1.561	1.525	− 2.3	5.4	1.474	−5.6	2.0	1.488	−4.7	2.1
Tryptophan	1.577	1.739	+10.3	1.4	1.700	+7.8	6.5	1.656	+5.0	2.1

The same consideration apply to the calculation of the "true" area of the added standard (A_T):

$$A_{D.n} = A_T = A_D - A_L C_L + (A_D - A_L C_L) C_D + (A_D - A_L C_L) C_D C_L + (A_D - A_L C_L) C_D^2 C_L \ldots \quad (3)$$

Substitution of the factors A_s and A_T by the series 2 and 3 yields

$$X_a = m_D \cdot \frac{(A_L - A_D C_D)(1 + C_L + C_L C_D + C_L^2 C_D + C_L^2 C_D^2 + C_L^3 C_D^2 \cdots)}{(A_D - A_L C_L)(1 + C_D + C_L C_D + C_L C_D^2 + C_L^2 C_D^2 + C_L^2 C_D^3 \cdots)} \quad (4)$$

$$X_a = m_D \cdot \frac{(A_L - A_D C_D)(1 + C_L C_D + C_L^2 C_D^2 \cdots) + (C_L + C_L^2 C_D + C_L^2 C_D^2 \cdots)}{(A_D - A_L C_L)(1 + C_L C_D + C_L^2 C_D^2 \cdots) + (C_D + C_L C_D^2 + C_L^2 C_D^3 \cdots)} \quad (5)$$

$$X_a = m_D \cdot \frac{(A_L - A_D C_D)(1 + C_L)(C_L C_D + C_L^2 C_D^2 + C_L^3 C_D^3 \cdots)}{(A_D - A_L C_L)(1 + C_D)(C_L C_D + C_L^2 C_D^2 + C_L^3 C_D^3 \cdots)} \quad (6)$$

$$X_a = m_D \cdot \frac{(A_L - A_D C_D)(1 + C_L)}{(A_D - A_L C_L)(1 + C_D)} = m_D \cdot \frac{A_L - A_D C_D + A_L C_L - A_D C_L C_D}{A_D - A_L C_L + A_D C_D - A_L C_L C_D} \quad (7)$$

Eqn. 7 allows the exact calculation of amino acid present in the sample at any degree of racemization of both the L-amino acid sample and the standard D-amino acid.

For testing both the accuracy and reproducibility of the method we spiked

$R = 1.5$			$R = 1$			$R = 0.7$			$R = 0.42$			$R = 0.25$		
C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)	C_f	Δ (%)	σ (%)
3.016	− 1.0	1.4	3.065	+ 0.7	1.1	3.069	+0.8	1.5	2.938	−3.6	1.0	3.028	− 0.5	0.9
2.392	+ 2.2	2.0	2.467	+ 5.3	4.0	2.443	+4.3	3.9	2.418	−3.1	5.5	2.273	− 3.1	9.6
2.518	+ 2.5	1.1	2.525	+ 2.2	0.4	2.597	+0.6	0.7	2.596	+0.5	2.2	2.685	− 2.0	14.5
2.385	+ 2.3	1.1	2.336	+ 0.2	1.0	2.488	+6.7	3.6	2.504	+7.4	2.0	2.912	+24.9	7.9
2.810	+ 3.4	1.9	3.019	+11.1	2.5	2.780	+2.3	0.7	2.932	−7.9	1.6	2.991	+10.1	2.3
2.199	+ 2.0	0.7	2.236	+ 3.5	0.6	2.246	+4.2	1.3	2.140	−0.75	2.8	2.182	− 1.2	1.2
2.866	− 4.2	2.6	3.187	+ 6.5	0.9	2.911	−2.7	0.8	2.847	−4.9	2.2	3.053	+ 2.0	5.3
2.382	− 0.1	0.3	2.542	+ 6.6	0.7	2.411	+1.1	0.4	2.419	+1.4	0.7	2.532	+ 6.2	1.3
2.227	− 2.0	1.2	2.284	+ 1.0	1.2	2.236	−1.1	1.3	2.263	±0	3.0	2.337	+ 3.2	3.4
2.156	+ 4.7	0.6	2.244	+ 8.9	0.8	2.162	+5.0	0.4	2.248	+9.1	2.8	2.251	+ 9.3	1.1
1.904	+ 0.4	1.2	1.919	+ 0.7	0.2	1.922	+1.3	2.4	1.853	−2.4	1.3	1.875	− 1.2	0.2
2.286	− 1.6	1.2	2.411	+ 3.7	1.7	2.283	−1.8	0.9	2.323	−0.1	1.0	2.426	+ 4.4	0.5
1.511	− 5.1	2.0	1.676	+ 5.2	0.7	1.611	+1.1	2.5	1.633	+2.5	0.2	1.605	+ 0.8	3.3
1.587	+ 1.7	0.3	1.617	+ 3.6	2.5	1.577	+1.0	1.7	1.579	+1.1	7.5	1.572	+ 0.7	10.7
1.769	+11.0	2.1	1.679	+11.7	5.8	1.664	+8.0	2.9	2.336	−1.8	—	1.578	+ 0.1	4.0

samples of a mixture of weighed amino acids with various amounts of the enantiomeric standard such that the nominal amount of amino acid to be quantitated varied from 5 to 95% in the mixture. Based on the known amount of standard added, the concentration of the L-amino acids in the mixture were calculated according to eqn. 7.

In Table II, the results are summarized together with standard deviations, based upon two separately derivatized samples and the absolute deviations from the nominal value. Obviously, the precision and accuracy are highest if the amounts of amino acid to be quantitated and the enantiomeric label are of the same order of magnitude. The deviation of the concentrations found remains within $\pm 2\%$ of the average value if the ratio of standard amino acid to sample is between 4 (80% D-amino acid) and 0.25 (20% D-amino acid).

Because of the oxidative degradation of tryptophan during derivatization, the response to a given amount of tryptophan is approximately 25% that of the other amino acids, which consequently decreases the accuracy of the determination of the ratio of D- to L-amino acid. By addition of 1 drop of mercaptoethane as antioxidant a four-fold increase in the tryptophan response is achieved. Because of its high volatility, mercaptoethane is readily removed after the esterification step. Another problematic amino acid is glycine, which is not optically active. D-Alanine was chosen as the most suitable standard, this time a non-identical compound. The response factor for glycine relative to D-alanine was 0.860.

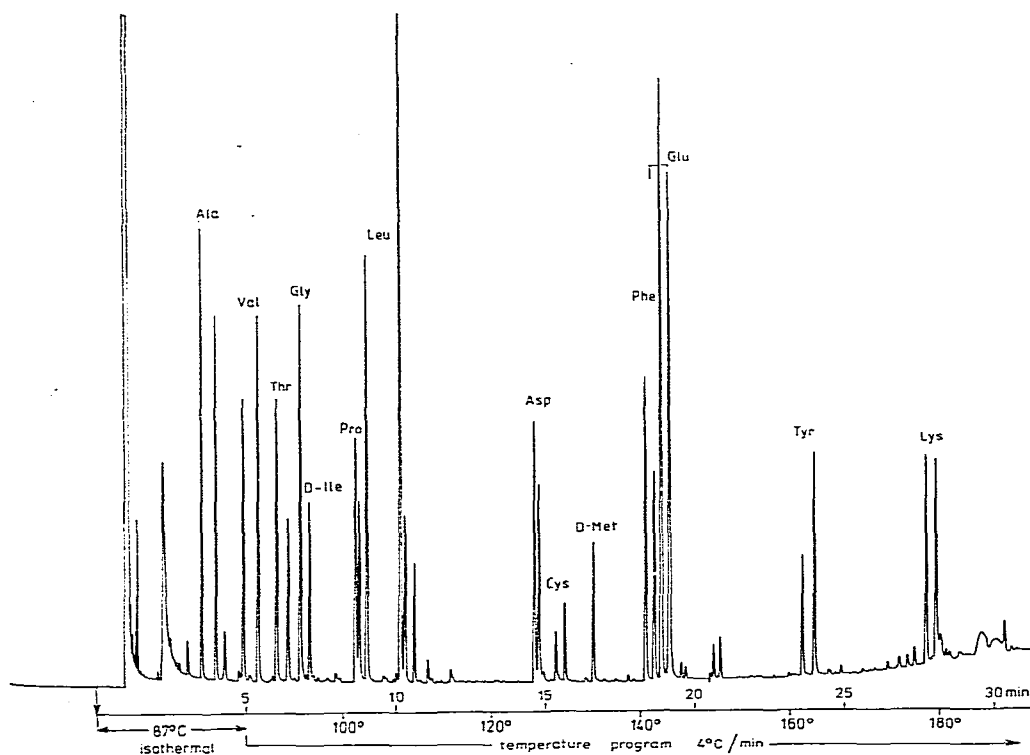

Fig. 2. Typical gas chromatogram obtained for amino acid analysis by enantiomer labelling of insulin B.

TABLE III

AMINO ACID ANALYSIS OF INSULIN-B-THIOSULPHONATE: COMPARISON OF ENANTIOMER LABELLING AND ION EXCHANGE PROCEDURE

Amino acid	*Theoretical composition**	*Enantiomer labelling*			*Racemization factor* (A_D/A_L)	*Ion-exchange chromatography*		
		*Composition**	*σ (%)* *(n=2)*	*Δ (%)*		*Composition**	*σ (%)* *(n=2)*	*Δ (%)*
Ala	2	1.95	0.4	− 2.5	0.0145	2.15	1.1	+ 7.5
Val	3	3.16	0.9	+ 8	0.154	2.81	1.2	− 9.5
Thr	1	1.02	±0	+ 2	0.007	1.00	1.0	± 0
Gly	3	3.09	1.8	+ 3	—	3.08	1.2	+ 2.7
Pro	1	1.08	±0	+ 8	0.027	2.04	2.5	+104 (?)
Leu	4	3.77	0.8	− 5.8	0.101	3.96	1.3	− 1
Ser	1	0.97	5.8	− 3	0.012	1.12	0.6	+ 12
Asp	1	1.27	5.6	+27	0.044	1.13	0.7	+ 13
Cys	2	2.10	6.7	+ 5	0.147	0.32	10.2	−168
Phe	3	2.70	±0	−10	0.020	2.97	1.0	− 1
Glu	3	3.04	0.2	+ 1.3	0.030	3.21	0.8	+ 7
Tyr	2	1.97	±0	− 1.5	0.018	2.05	0.9	+ 2.5
Lys	1	0.995	2.2	− 1	0.013	1.05	1.0	+ 5
Arg	1	n.d.**			—	1.11	1.2	+ 11
His	2	n.d.			—	2.02	1.0	+ 1

* No. of amino acids present per molecule.
** n.d. = not determined.

We applied the method of enantiomer labelling to the quantitative determination of the amino acid composition of bovine insulin B chain thiosulphonate. Fig. 2 depicts a typical chromatogram. The results are summarized in Table III. The D-amino acids found in the sample result from racemization during hydrolysis and are not constituents of the natural insulin[10]. The high error in the proline determination by the ion-exchange procedure may be explained by bacterial digestion of L-proline in the standard used for calibration. We noted that our D-standards were not decomposed by bacterial action over a month. D-Amino acids would therefore be preferable also as standards for the ion-exchange procedure. The low value of cystine obtained by the ion-exchange procedure is presumably due to incomplete oxidation of cysteine during handling of the sample in the presence of air. This problem is eliminated in the gas chromatographic procedure. Both the D-cysteine added as an internal standard and the cystine/cysteine from the sample are converted during derivatization in equal yields into cysteine, which is chromatographed as the S-pentafluoropropionyl derivative.

For calculation of threonine, the areas of the two *allo*-enantiomers were added to the areas of the corresponding *threo*-antipodes. As a consequence, no additional correction for mutual contribution of racemized D- and L-enantiomers to the respective peak areas was necessary.

Histidine and arginine were not determined in this investigation because of low yields in their derivatization. The difficulties arise from the poor solubility of histidine in the esterification medium and incomplete acylation of the guanidino group of arginine[11]. Separately prepared samples of N^{α}-pentafluoropropionyl N^{im}-

ethoxycarbonylhistidine isopropyl ester and N^{α},N^{G},N^{G}-tripentafluoropropionylarginine isopropyl ester could be readily chromatographed on the columns employed. The derivatization yields during the analysis of insulin B were, however, insufficient for quantitation. Conditions for an improved derivatization of these amino acids are under investigation.

CONCLUSIONS

Enantiomer labelling is a fast, accurate and sensitive method for the quantitation of chiral compounds amenable to separation of the enantiomers. The speed and sensitivity of this procedure as applied to the quantitative analysis of amino acids are higher than those of the widely used ion-exchange procedure. The accuracy is generally of similar magnitude, and for some amino acids is considerably higher.

Enantiomer labelling is not limited to quantitation of an optically pure enantiomer present in a biological sample but can also be applied to racemates. In such a case, either of the two enantiomers may be added as the internal standard; for quantitation of an optically pure compound the racemic mixture can also be used as internal standard. However, the accuracy of a quantitation is higher, the more the enantiomeric composition of the compound to be quantitated differs from that of the added standard. Further improvements to the method can probably be achieved by using a selective detector, *e.g.*, a nitrogen detector, electron-capture detector or mass spectrometer in the single-ion monitoring mode.

The method is especially useful if only minimal amounts of compound are available, *e.g.*, for the analysis of proteins isolated from biological samples. In such cases addition of the enantiomeric standard may even serve as a carrier similar to isotopic dilution analysis.

Potential further applications are the quantitative determination of adrenergic compounds of the α-phenyl-β-aminoethanol type and epinephrine metabolites in biological samples or the quantitative determination of amino acids in biological fluids, extraterrestrial materials, sediments or minerals.

For routine analysis of amino acids by enantiomer labelling, use of an electronic integrator and an automatic sampling device is indispensable. The time required for one analysis is reduced to about half that of the method of Spackman *et al.*[1]. As no dedicated instruments are required, higher flexibility and lower costs are expected.

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