Vol. 86, No. 4, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1979 Pages 995-1001

CHIROPTICAL STUDIES OF FLUORESCAMINE LABELED AMINO ACIDS

K.L. Kovács

Institute of Biophysics, Biological Research Center of Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521, Hungary

Received December 15,1978

SUMMARY

Absorption and circular dichroism studies of fluorescamine condensation products with α-amino acids, dipeptides and phenylethylamine in the 300-450 nm region are reported. The results make a major revision of the previously suggested rule for absolute configuration determination necessary.

INTRODUCTION

Fluorescamine, 4-phenylspiro /furan-2(3H),1'-phthalan/--3,3'-dione, has been widely used recently for sensitive and fast fluorometric assay /1-4/, and labeling of primary as well as secondary amines /5,6/. The condensation products of fluorescamine and optically active amines (e.g. amino acids) were also found to possess characteristic chiroptical properties /7,8/. Toome and coworkers detected several Cotton effects in the 200-400 nm wavelength region and inferred a simple chiroptical rule for the determination of absolute configuration of any unknown amino acid containing primary amine group. According to this rule "the first Cotton effects (around 385 nm) of the chromophores derived from L-amino acids and fluorescamine are positive and the second Cotton effects (around 325 nm) are negative" /7/. The circular dichroism (CD) spectra of D-amino acid-derived chromophores are mirror images of those of the L-amino acids. In our UV absorption and CD studies on the same derivatives several amino acids have been found which do not obey the above chiroptical rule. Absolute configuration of an amino acid can be determined from the CD spectra of their fluorescamine reaction products but not in the way cited.

0006-291X/79/040995-07$01.00/0

EXPERIMENTAL

1. Reagents

Fluorescamine (Fluram^{R}) was purchased from Hoffmann La Roche Inc., Nutley, N.J., USA. Analytical grade dioxane (Reanal, Hungary) was purified by fractioned distillation over sodium. Borate buffer (0.05 M, pH 8) was prepared according to Clark and Lubs /9/. Amino acids were obtained from different sources as indicated in Table I and used without further purification.

2. Methods

Samples for spectroscopic investigations were prepared according to Toome et al. /7/ with the following modifications: 2 ml of 2 mM solution of fluorescamine were added to 2 ml of 0.5 mM vigorously stirred solution of an amino acid. The reaction mixture was measured directly in 0.2-1 cm quartz cuvettes (selected to obtain 0.5-1 optical density at the absorption maximum). Absorption spectra were recorded on a Unicam SP 1800 spectrophotometer, CD measurements were performed on a JASCO 40c dichrograph. The spectral changes in the wavelength region between 450 and 300 nm are discussed here. The data are the average of at least three repetitions for each compound.

RESULTS AND DISCUSSION

Fluorescamine reaction has several benefits when compared with other chiroptical methods:

a./ the reagent is stable at room temperature, it can be dissolved in common, water miscible organic solvents;

b./ the reaction is easy to carry out and is very sensitive, the pyrrolinone-type chromophore needs no further treatment /isolation, etc./, the reaction product is stable for hours;

c./ fluorescamine forms its condensation compounds within microseconds which can be investigated at convenient wavelength range. The unreacted fluorescamine does not interfere with the measurement of the chromophoric derivatives (Fig. 1/a).

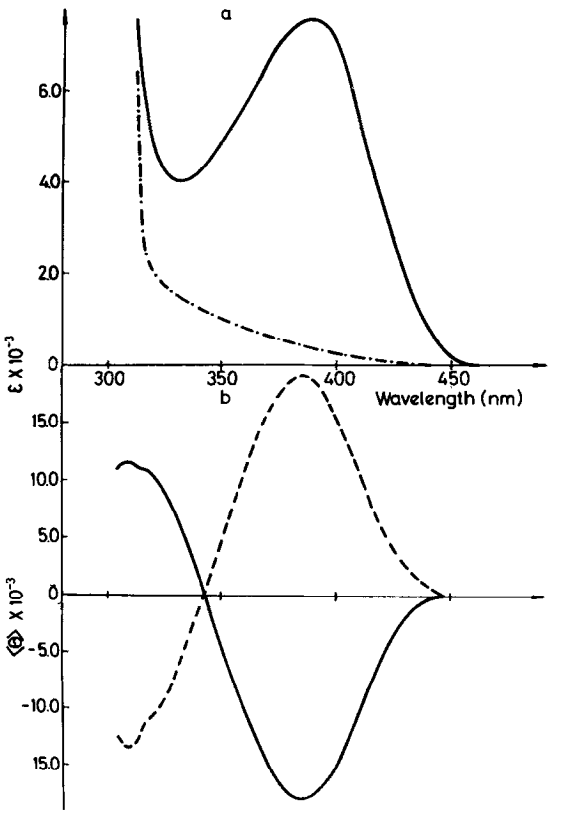

Figure 1. Absorption /a/ and CD /b/ spectra of fluorescamine derivatives of tryptophan isomers. -.-.- unreacted fluorescamine, —— D-tryptophan, ----L-tryptophan.

Absorption spectra of fluorescamine labeled amino acids are similar in shape, small shifts of the absorption maximum (387 ± 3 nm) and intensity changes are only detectable (Table I). Difference absorption spectra (i.e. fluorescamine + buffer is used as reference) reveal a low intensity band around 320 nm as well (Fig. 2/a). The latter may have its origin in secondary amine contamination /4/ in our α-amino acid sample or it is related to the transition corresponding to the short wavelength Cotton effect. The small amplitude of this transition does not make possible a quantitative evaluation under the employed experimental conditions.

α-amino acids as well as dipeptides produced two Cotton effects, discussed as first (∿385 nm) and second (∿320 nm) by

Table I.

Absorption and CD data of fluorescamine labeled amino acids

Amino acid	Source	Absorption		Circular dichroism			
				1st		2nd	
		ε^a	λ^c_{max}	$\langle\theta\rangle^b \times 10^{-3}$	λ^c_{max}	$\langle\theta\rangle^b \times 10^{-3}$	λ_{max}
L-Leucine	Fluka	4313	387.0	+ 7.20	385.0	-14.10	322.0
D-Leucine	Fluka	4125	388.0	- 7.00	385.0	+13.20	322.0
L-Cysteine	Calbiochem.	6700	387.0	+ 3.80	395.0	- 4.00	324.0
D-Cysteine	Calbiochem.	6625	387.0	- 4.20	395.0	+ 2.80	323.0
L-Histidine	Fluka	4423	386.5	+ 9.30	386.5	-11.38	322.0
D-Histidine	Fluka	4828	386.5	-10.25	386.5	+14.00	321.5
L-DOPA	Sigma	3300	387.0	+ 3.12	385.0	- 2.24	320.0
D-DOPA /partly oxidized/	Sigma	10300	386.0	-13.12	386.0	+ 9.20	320.0
L-Phenylalanine	Fluka	3781	387.0	+22.90	384.0	-13.80	320.5
D-Phenylalanine	Fluka	3250	388.0	-17.00	383.0	+10.75	322.0
L-Tryptophane	Merck	7500	388.5	+18.78	386.5	-14.40	311.0sh[d]
D-Tryptophane	Merck	7125	388.0	-19.55	388.0	+11.88	311.5sh[d]
L-Tyrosine	Merck	4305	388.0	+ 6.90	385.5	- 3.79	321.5
D-Tyrosine	Merck	4556	388.5	- 6.74	387.0	+ 3.38	322.0
D-Methionine	Calbiochem.	5500	389.0	- 0.40	390.0	+ 3.60	318.0
L-Threonine	Sigma	8000	386.0	- 2.64	390.0	- 7.40	321.0
D-Threonine	Sigma	8500	387.0	+ 2.74	389.0	+ 7.61	318.5
L-Glutamic acid	Fluka	2500	388.0	- 1.80	392.0	- 4.88	321.5
D-Glutamic acid	Fluka	2188	388.0	+ 1.43	392.5	+ 3.93	321.0

Table I. (continued)

L-Arginine	Fluka	9300	388.0	- 2.60	388.0	- 3.48	318.0
D-Arginine	Fluka	9500	388.0	+ 2.68	388.0	+ 3.36	318.0
L-Alanine	Fluka	4756	387.5	- 2.16	386.5	- 6.95	318.5
L-Isoleucine	Fluka	6300	390.0	- 4.32	388.0	- 7.92	315.0
D-Isoleucine	Fluka	6600	390.0	+ 5.76	390.0	+ 8.40	318.0
L-Valine	Fluka	4375	389.0	- 3.90	390.5	- 5.42	315.0
D-Valine	Fluka	4275	390.0	+ 4.00	390.5	+ 5.19	315.0
L-Aspartic acid	Fluka	2000	385.0	- 2.85	390.0	- 2.40	320.0
D-Aspartic acid	Fluka	1750	386.0	+ 2.25	388.0	+ 1.80	319.0
L-Lysine	Fluka	5906	387.0	- 3.83	384.0	- 5.86	319.0
D-Lysine	Fluka	5906	386,5	+ 3.88	385.0	+ 5.75	318.5
D-amino-n--butyric acid	Calbiochem.	3344	390.0	+ 2.62	388.5	+ 1.44	317.5
D-Asparagine	Calbiochem.	4000	385.0	+ 0.52	380.0	+ 1.92	319.0
D-Serine	Calbiochem.	6500	386.0	+ 3.04	385.0	+ 2.08	319.0
L-Tyr-L-Tyr	Nutritional Biochem.Co.	6563	385.5	+ 5.84	379.0	-10.63	325.5
L-Leu-L-Tyr	"	8119	385.0	- 5.62	385.5	- 4.97	319.5
/-/-α-phenyl-ethyl-amine	Norse	10600	387.0	-17.60	387.0		
/+/-α-phenyl-ethyl-amine	Norse	10600	387.0	+17.40	387.0		

/a/ in $mole^{-1}cm^{-1}$. /b/ in deg cm^2 $decimol^{-1}$. /c/ in nanometers. /d/ shoulder.

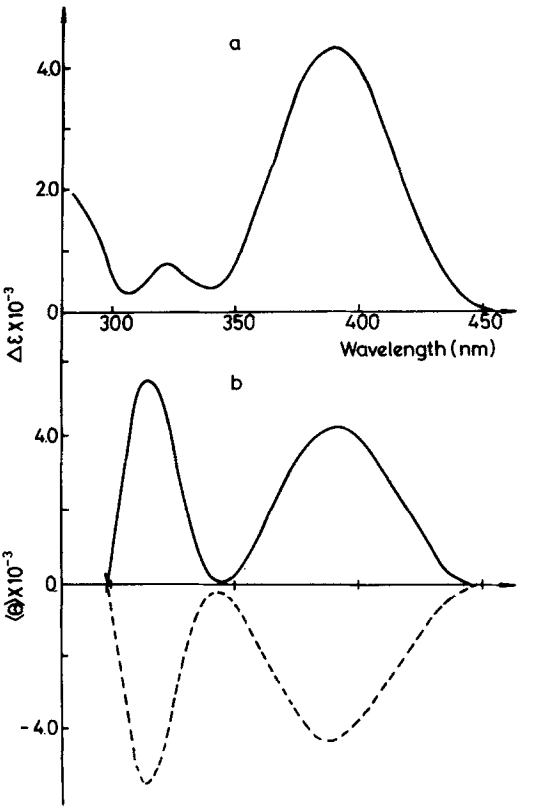

Figure 2. Difference absorption /a/ and CD /b/ spectra of fluorescamine derivatives of valine isomers. —— D-valine, ----L-valine.

Toome et al. /7/. Phenylethylamine optical isomers show only the first transition indicating that interaction between the chromophore and the carboxyl group of an amino acid is responsible for the second CD band.

The studied amino acids can be devided into two groups (Table I). About half of them fit into the chiroptical rule described in ref. 7 (Fig. 1/b) but the other half does not: the two Cotton effects have the same sign (Fig. 2/b). Examples of the second group have also been found by Toome et al. /7/ (alanine and isoleucine) although only alanine is discussed. Other amino acids in group II. (valine, serine, asparagine, aspartic acid, lysine, and amino-n-butyric acid) were tested only in the present study. Our measurements are in apparent contra-

diction with that of Toome and coworkers in the case of threonine, arginine and glutamic acid. Two out of these three amino acids were obviously difficult to measure on their instrument (see Table I in ref. 7) which may explain the different results. It is interesting to note that while leucine, tyrosine, and even tyrosyl-tyrosine belong to group I (i.e. the two CD bands have opposite signs) in leucyl-tyrosine the sign of the 385 nm Cotton effect is reversed. D-leucyl-L-tyrosine and L-leucyl-L-tyrosine were not mirror symmetric but this puzzle was solved by gaschromatographic analysis and leucine-aminopeptidase digestion which disclosed that our D-leucyl-L-tyrosine sample contained in fact at least 90% of L-leucyl-L-tyrosine.

From the data it is concluded that the chiroptical rule /7/ based on the first CD band of these compounds is not generally applicable. The absolute configuration of an amino acid, however, can easily be determined from the second Cotton effect. Without exception the second CD bands of the tested L-amino acids are negative and for the D-amino acids they are positive. The long wavelength CD band is useful in stereochemical assignment and in checking optical purity of primary amines if standard sample of known configuration is available.

ACKNOWLEDGEMENTS

I thank Dr. J. Soós of Institute of Biophysics, BRC for the gaschromatographic analyses and Mrs. R. Verebélyi for skillful technical assistance.

REFERENCES

1. Udenfriend, S., Stein, S., Bohlen, P., Dairman, W., Leimgruber, W., and Weigele, M. /1972/ Science, 178, 817-872.
2. Weigele, W., De Bernardo, S. L., Tengi, J. P., and Leimgruber, W. /1972/ J. Amer. Chem. Soc., 94, 5927.
3. Nakai, N., Lai, C. Y., and Horecker, B. L. /1974/ Anal. Biochem., 58, 563.
4. Toome, V., and Manhart, K. /1975/ Anal. Letters, 8, 441-448.
5. Handschin, U. E., and Ritschard, W. J. /1976/ Anal. Biochem., 71, 143-155.
6. Stephens, R. E. /1978/ Anal. Biochem., 84, 116-126.
7. Toome, V., Wegrzynski, B., and Reymond, G. /1976/ Biochem. Biophys. Res. Commun., 69, 206-211.
8. Toome, V., Wegrzynski, B., and Dell, J. /1976/ Biochem. Biophys. Res. Commun., 71, 598-602.
9. Clark, W. M., and Lubs, H. A. /1916/ J. Biol. Chem., 25, 479-484.