Wetlaufer, D. B. (1962), *Advan. Protein Chem. 17*, 303.
Williams, E. J., and Foster, J. F. (1959), *J. Am. Chem. Soc. 81*, 865.
Williams, E. J., Herskovits, T. T., and Laskowski, M., Jr. (1965), *J. Biol. Chem. 240*, 3574.
Williams, E. J., and Laskowski, M., Jr. (1965), *J. Biol. Chem. 240*, 3580.
Yanari, S., and Bovey, F. A. (1960), *J. Biol. Chem. 235*, 2818.
Yasunobu, K. T., and Dandliker, W. B. (1957), *J. Biol. Chem. 224*, 1065.

On the Alleged Identification of Monoiodohistidine by Ultraviolet Spectrophotometry*

Caroline T. Holloway,† R. P. M. Bond, I. G. Knight, and R. B. Beechey

ABSTRACT: 4(5)-Monoiodohistidine (in 0.1 M phosphate buffer at pH 7.4) has no absorption maximum in the ultraviolet above 210 nm. Inorganic iodide exhibits a maximum at 227 nm with ϵ 13,500. Inorganic triiodide is a frequent contaminant of solutions of iodide and has a maximum at 287 nm (ϵ 40,000). A recently published ultraviolet spectrum purporting to be that of monoiodohistidine exhibits maxima at *ca.* 230 and *ca.* 280 nm.

In a recent paper in this journal (Perlgut and Wainio, 1967), an ultraviolet spectrum was published purporting to be that of a sample of monoiodohistidine which had been eluted, as a standard, from a G-10 Sephadex column. This was compared with an essentially identical spectrum of a material eluted from the same column during chromatography of a crude mitochondrial extract. These spectra exhibited maxima at about 230 and 280 nm, the latter peak being low and broad. Paper chromatography of the materials showing this ultraviolet absorption, and of other fractions from the column, showed, after treatment of the chromatograms with the FFCA[1] spray reagent, only one spot. This spot had the same mobility as inorganic iodide. This finding was attributed to the decomposition of organic iodides on the paper.

Chromatography of the "standard" monoiodohistidine and of mitochondrial extract was then repeated on another G-10 Sephadex column, the effluent being monitored by following the ultraviolet absorption at 230 nm. It was stated that "this method of detecting the peaks eliminated the interference by inorganic iodide with the monoiodohistidine peaks, as detected by the FFCA method."

We wish to point out: (i) that 4(5)-monoiodohistidine (in 0.1 M phosphate buffer at pH 7.4) shows no absorption maximum above 210 nm, but only end absorption; and (ii) that iodide ion absorbs[2] at 227 nm (ϵ 13,500; Mellor's Treatise, 1956) and that the spectrum shown in Figure 2 of the paper cited is essentially the same as that of iodide ion containing (as is common with iodide solutions exposed to air; Brode, 1926) a trace of triiodide ion (I_3^-) (λ_{max} 287 nm (ϵ 40,000) (Brode, 1926; Autrey and Connick, 1951)).

In view of these facts, the use of absorptiometry at 230 nm to detect monoiodohistidine without interference from iodide ion is not a procedure which can be generally recommended. In addition, it is clear from the results of both paper chromatography and ultraviolet spectroscopy that the only material which these authors detected from their Sephadex column was inorganic iodide. We believe, therefore, that these "results" do nothing to reestablish the authors' previously controverted (Holloway *et al.*, 1967) claim that monoiodohistidine occurs in mitochondrial extracts.

References

Autrey, A. D., and Connick, R. E. (1951), *J. Am. Chem. Soc. 73*, 1842.
Brode, W. R. (1926), *J. Am. Chem. Soc. 48*, 1877.
Burak, I., and Treinin, A. (1963), *Trans. Faraday Soc. 39*, 1481.
Halmann, M., and Platzner, I. (1964), *Proc. Chem. Soc.*, 261.

* From the Milstead Laboratory of Chemical Enzymology, Shell Research Ltd., Sittingbourne, Kent, England. *Received March 31, 1967.*

† Milstead research fellow.

[1] Abbreviation used: FFCA, ferric ferricyanide–arsenious acid.

[2] There is, of course, a considerable literature on the ultraviolet absorption of inorganic iodide. Recent work has been mostly on establishing the nature of the transition involved. For leading references see, *e.g.*, Halmann and Platzner (1964) and Burak and Treinin (1963).

Holloway, C. T., Bond, R. P. M., Knight, I. G., and Beechey, R. B. (1967), *Biochemistry 6*, 19.
Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry (1956), Supplement II, Part I, Briscoe, H. V. A., Eldridge, A. A., and Dyson, G. M., Ed., London, Longmans Green, p 934.
Perlgut, L. E., and Wainio, W. W. (1967), *Biochemistry 6*, 15.

The Conformational Transitions of Uncharged Poly-L-lysine. α Helix–Random Coil–β Structure*

Betty Davidson and Gerald D. Fasman†

ABSTRACT: The heat-induced transition of poly-L-lysine, α helix–random coil–β form, has been studied, mainly by optical rotatory dispersion (ORD). Below T_β, the transition has the properties of a reversible α-helix–random coil equilibrium. Above T_β, the transition is not reversible during the period of the measurements; it proceeds at a rate dependent upon polymer concentration, temperature, pH, and solvent. Evidence is presented that above T_β the thermal transition represents the conversion of α-helical poly-L-lysine to an intermediate random coil and finally to an intermolecular antiparallel β structure. At 50° the β form is the most stable of the three conformations of poly-L-lysine, and its conversion from the helix is essentially complete. At 4° the α helix is the most stable form; however, β–α conversion is very slow. In comparison to H_2O, the β form is destabilized by 15% ethylene glycol and is probably stabilized by 0.2 M NaCl. The α helix is destabilized by 2.0 M LiBr and stabilized by 50% methanol. The different behavior of the two conformations with respect to temperature and solvent is consistent with the proposal that α-helical poly-L-lysine is stabilized largely by intraamide hydrogen bonds while β poly-L-lysine owes a large part of its stability to hydrophobic interactions between lysyl residues.

Of the three conformations known to exist in proteins and polypeptides (the α helix, β-pleated sheet, and random coil), the β structure (the pleated-sheet structure) in solution has been the least well characterized. The pleated-sheet structures were first studied and characterized by means of X-ray diffraction (Meyer and Mark, 1928; Astbury and Woods, 1930; Astbury and Marwick, 1932; Pauling *et al.*, 1951; Pauling and Corey, 1951), and the results were correlated with infrared absorption data (Astbury *et al.*, 1948; Ambrose and Elliot, 1951). These early studies classified the β structure as an intermolecularly hydrogen-bonded pleated sheet, further aggregated by three-dimensional stacking (Pauling and Corey, 1951; Marsh *et al.*, 1955). The polypeptide chains have either the same (parallel) or alternating (antiparallel) direction (Pauling and Corey, 1951). A third type of β structure, the intramolecularly hydrogen-bonded "cross β," has also been described (Dickerson and Bailey, 1935; Parker and Rudall, 1957). The parallel and antiparallel forms have been considered in a theoretical treatment of the amide I and II infrared absorption bands (Miyazawa and Blout, 1961).

Both the α-helical and the β forms have been found to be energetically favorable conformations for the polypeptide chain (Pauling *et al.*, 1951; Pauling and Corey, 1951; Ramachandran *et al.*, 1963a,b; Némethy and Scheraga, 1965; De Santis *et al.*, 1965). The preferential formation of the β structure in some high molecular weight synthetic polypeptides was found to depend on the presence of bulky substituents or hetero atoms on the β carbon of the amino acid side chain (Bloom *et al.*, 1962; Blout, 1962).

Early studies of the β structure were carried out on samples in the solid state owing in part to the insolubility of this aggregated conformation in aqueous solution. Recently, this difficulty has been overcome and the study of the β conformation in aqueous solution has been reported (Davidson *et al.*, 1966; Sarkar and Doty, 1966). Optical rotatory dispersion (ORD)

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