The Effect of Oxidation and pH on the Molecular Weight of Human Growth Hormone, Estimated by Gel Filtration

By P. FØNSS-BECH

Department of Biochemistry, Nordisk Insulinlaboratorium, Gentofte, Denmark

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The molecular-weight estimation of protein by Sephadex gel filtration (Andrews & Folley, 1963; Andrews, 1964, 1965, 1966) has been used for following the change in molecular weight of oxidized human growth hormone.

The hormone used was prepared in this Laboratory from fresh frozen human pituitaries by the author's modification of the method of Wilhelmi (1961). The gland residue from extract A was suspended in water at pH 9 and extracted twice. The combined extracts were adjusted to pH 7.0 and solid $(\text{NH}_4)_2\text{SO}_4$ was added to give a final concentration of 2M. After centrifuging, the precipitate was

suspended in water, dialysed salt-free against water and freeze-dried. This fraction was extracted three times with 0.1M-glycine-0.1M-NaCl buffer, pH 5.8. After centrifuging, the combined supernatants were dialysed, freeze-dried and redissolved in the glycine buffer and chromatographed on a Sephadex-G-100 column with the same glycine buffer for the elution. The peak containing the growth hormone was dialysed salt-free and freeze-dried. The whole preparation was carried out in a cold room at 3°.

The purity of the growth hormone was checked by determination of the N- and C-terminal amino acids. Determination of the N-terminal amino acid sequence (Fraenkel-Conrat, Harris & Levy, 1958) gave Phe-Pro-Thr-Ileu-Pro, in agreement with that found by Liu, Dixon & Li (1964) and Li, Liu & Dixon (1966). Zone electrophoresis on starch (Fønss-Bech & Li, 1954), free electrophoresis and disk electrophoresis (Ornstein, 1964, slightly modified) was also used. Compared with the original Wilhelmi preparation, the present preparation has only two zones instead of five when run on disk electrophoresis and it is about 30% more active as determined by radio-immunological assay (O. Trygstad, personal communication). The oxidation of the growth hormone was carried out by the method of Craig (1956).

The standard curve for molecular-weight determination was made according to the method of Andrews (1964) with cytochrome c (from horse heart), myoglobin (from sperm whale), trypsin inhibitor (from soya bean), chymotrypsinogen and serum albumin (bovine) as standards. Plots of elution volumes, V_e , against $\log(\text{molecular weight})$ gave a straight line over the molecular-weight range from about 10^4 to 6.7×10^4 .

Andrews (1966) claims that there is a correlation between the amounts of growth hormone used and the molecular weight found, but it has not been possible to confirm this (see Fig. 1a). There is therefore no reason for extrapolation to infinite dilution. The average molecular weight at pH 7.5 for the monomer human growth hormone is $2.2 \times 10^4 \pm 10\%$ (the limits are those given by Andrews, 1966). Fig. 1(b) shows that the human growth hormone contains a small amount of a dimer but no higher polymerization products at pH 7.5; the oxidized hormone, on the other hand, has a high

Fig. 1. Molecular weights of human growth hormone estimated by Sephadex G-100 filtration. (a) Concentration-dependence at pH 7.5. (b) Elution diagram for human growth hormone at pH 7.5 (4.0 mg. sample). (c) and (d) Elution diagrams for oxidized human growth hormone at pH 7.5 and pH 8.5 (2.3 mg. and 2.1 mg. samples respectively).

peak of a probably pentamer component and a small peak of a trimer at the same pH (Fig. 1c). After changing the pH to 8.5 but still with tris-HCl buffer (Fig. 1d) the oxidized growth hormone shows a different proportion between the two polymers, with a shift to a lower degree of polymerization.

At the present time it is not possible to elucidate the reason for the higher polymerization after oxidation or the shift to a lower degree of polymerization on increasing the pH.

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- Andrews, P. (1964). *Biochem. J.* **91**, 222.
 Andrews, P. (1965). *Biochem. J.* **96**, 595.
 Andrews, P. (1966). *Nature, Lond.*, **209**, 155.
 Andrews, P. & Folley, S. J. (1963). *Biochem. J.* **87**, 3r.
 Craig, L. C. (1956). In *Ciba Found. Colloq.: Endocrinology* vol. 9, p. 107. Ed. by Wolstenholme, G. E. W. & O'Connor, C. M. Boston, Mass.: Little, Brown and Co.
 Fønss-Bech, P. & Li, C. H. (1954). *J. biol. Chem.* **207**, 175.
 Fraenkel-Conrat, H., Harris, J. I. & Levy, A. L. (1958). *Meth. biochem. Anal.* **2**, 359.
 Li, C. H., Liu, W.-K. & Dixon, J. S. (1966). *J. Amer. chem. Soc.* **88**, 2050.
 Liu, W.-K., Dixon, J. S. & Li, C. H. (1964). *Biochim. biophys. Acta*, **93**, 428.
 Ornstein, L. (1964). *Ann. N.Y. Acad. Sci.* **121**, 321.
 Wilhelmi, A. E. (1961). *Canad. J. Biochem. Physiol.* **39**, 1659.

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Reduced Nicotinamide-Adenine Dinucleotide as an Allosteric Effector of Citrate-Synthase Activity in *Escherichia coli*

By P. D. J. WEITZMAN

Department of Biochemistry, University of Leicester

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It has been shown (Weitzman, 1966a) that the citrate synthase (EC 4.1.3.7) of *Escherichia coli* differs from the enzyme purified from mammals and yeast in being insensitive to inhibition by ATP: instead, the enzyme is powerfully inhibited by NADH, and this is reversible with the concentration of acetyl-SCoA. It is the purpose of this communication to present evidence which indicates that, under certain circumstances, the inhibitory action of NADH may be abolished without loss of the catalytic activity of the enzyme, and that such circumstances may also produce conformational changes in the enzyme protein.

Except where otherwise stated, assays were performed by the polarographic measurement of CoASH production (Weitzman, 1966b) in buffer, of composition 20mM-tris-10mM-MgCl₂-1mM-EDTA, at pH 8.0 in the presence of 0.2mM-oxaloacetate and 0.16mM-acetyl-SCoA. Citrate synthase was purified from *E. coli* strain 114-IL⁺ (Kornberg, 1966) to a specific activity of 30 μ moles of CoASH produced/min./mg. of protein.

As shown in Fig. 1 (curve C) the inclusion of 0.2mM-NADH in the above assay system virtually abolished enzyme activity at pH values of less than 7.5, but had progressively less effect as the pH was raised; at pH 8.5 only 52% inhibition was observed. At pH 9.2 the enzyme was no longer inhibited by

NADH, although (Fig. 1, curve A) the catalytic activity at this pH, in the absence of NADH, was still 50% of that observed at the optimum value of pH 8.0. Titration of NADH confirmed that it has no dissociating group in the pH region 8-9. Thus it is most probable that the enzyme itself is modified by the change of pH and that the regulatory and catalytic sites on the enzyme are not identical.

Since a change in the ionic environment appears to modify the regulatory site, the effect of increased salt concentration was examined at pH values at which the enzyme normally showed strong inhibition by NADH. It was found that, at pH 8.0, the presence of 0.2M-KCl in the assay medium left the catalytic activity essentially unchanged but completely abolished the sensitivity to NADH (Fig. 1, curve D). Moreover, although the activity of the enzyme at this pH was virtually the same in the absence or presence of 0.2M-KCl, the presence of this salt markedly affected the activity of the enzyme at other pH values, and particularly in that region (pH 8.0-8.5) at which, in the absence of KCl, NADH ceases to exert its inhibitory effect (Fig. 1, curves A and B). Similar effects were observed with other salts (NaCl, KNO₃, Na₂SO₄) but, at 0.2M, these were less effective than KCl in overcoming the inhibition; likewise, the inhibitory action of NADH