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Human Pituitary Growth Hormone. Physicochemical Investigations of the Native and Reduced-Alkylated Protein*

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ABSTRACT: Results of investigations into the biological and physicochemical properties of native, reduced-tetra-*S*-carbamidomethylated, and reduced-tetra-*S*-carboxymethylated human pituitary growth hormone are presented. While both derivatives appear to retain lactogenic activity, only the carbamidomethylated product retains growth-promoting potency. Measurements of chemical composition, molecular weight, viscosity, spectrophotometric titration of tyrosyl groups, and circular dichroism have failed to uncover any significant differences between the two derivatives. In addition, differences between either derivative and the native hormone appear to be very small. Accordingly, we conclude that the disulfide bonds in this molecule are not necessary for the manifestation of biological activity nor are they required for the formation of the secondary and tertiary structure. However, investigation of the relative rates of proteolysis by trypsin would indicate that the presence of these bonds does serve to stabilize the molecular architecture against perturbing forces. The carbamidomethylated derivative is digested about 1.5 times as fast as the native, while the carboxymethylated product, under identical conditions, is digested at 2.5–3 times the rate of the native hormone.

The general acceptance of the hypothesis that the native conformation of a protein is mostly a result of noncovalent intramolecular forces arising from its amino acid sequence (Epstein *et al.*, 1963; Anfinsen, 1964) raises an interesting question as to the specific role of disulfide bonds in either achieving and/or stabilizing these conformations by strategic placement of a few nonpeptidic covalent links. In addition to their functioning as structural restraints, there is also the question of whether some disulfide bonds may be intrinsically involved in the active site(s) of the proteins which contain them. The general approach to these problems is the application of a selective chemical modification followed by biological and physicochemical evaluation of the derivative.

Experimentally, disulfide modification by the use of harsh and relatively nonspecific oxidative techniques or reductive cleavage in the presence of strong denaturing agents makes it very difficult if not impossible to determine whether or not subsequent changes in either conformation and/or biological activity are solely a consequence of disulfide cleavage. Ideally, one must be able to separate those effects resulting purely from the modification employed from those effects resulting purely from the conditions under which the modification was carried out. This ideal would seem to be approachable only in those cases where the conditions of modification are so passive that they do not in themselves contribute to the effects of modification.

It has been demonstrated that the two disulfide bonds in HGH[1] may be quantitatively reduced at pH 8.1 with dithiothreitol in the complete absence of denaturant (Bewley *et al.*, 1968). The reduced product, following alkylation of the thiol groups with iodoacetamide was found to retain essentially full biological activity as previously reported by Dixon and Li (1966). Subsequently, it was found (Bewley, 1968) that when iodoacetic acid was used as the alkylating agent instead of iodoacetamide, the product displayed no growth-promoting activity. In view of the very mild reaction conditions employed these two reduced-alkylated derivatives, along with the native hormone, provide an interesting system for studying the relationship between structure and activity and the influence of the disulfide bonds on both. Such a study is even more attractive with these molecules since the amino acid sequence of HGH is known (Li *et al.*, 1966)

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[1] Abbreviations used are: HGH, native human growth hormone; RCAM, reduced tetra-*S*-carbamidomethylated HGH; RCOM, reduced tetra-*S*-carboxymethylated HGH.