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SOME COMPARATIVE OBSERVATIONS ON THE ELECTROSTATIC AND HYDRODYNAMIC BEHAVIOR OF BOVINE, HUMAN AND MONKEY GROWTH HORMONES*

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Recent investigations have disclosed some interesting similarities between growth hormones isolated in pure form from human and monkey pituitary glands[1]; the corresponding bovine hormone, however, differs significantly with respect to amino acid composition, molecular weight, and the number of peptide chains[2]. Since it appears desirable to extend the comparison, we wish to report on some aspects of the gross molecular configuration of the three proteins, based upon their electrostatic[3] and hydrodynamic[4] properties.

Since complete titration curves could not be secured, owing to the limited solubility of the bovine material and to the scarcity of primate preparations, the electrostatic parameters were estimated from a study of the ionization properties of tyrosine residues only. Absorption spectra of the growth-hormone solutions in 0.1 *N* KCl were recorded at 25° in the range of 280–300 mμ, the pH being varied between 9 and 13.5; allowance was made for background absorbancy, extrapolated from the region of 330–370 mμ as discussed by BEAVEN AND HOLIDAY[5]. The tyrosine content of each hormone was recalculated from these measurements (Table I). Spectrophotometric values were always greater than the earlier values obtained by means of the paper-dinitrophenylation method[1,6]; the former values appear to be the more reliable, however, since the latter procedure has well known limitations with respect to the analysis of tyrosine. Spectral changes associated with variation of the pH were reversible; they were also instantaneous, except at pH 13.5 where a slight increase in absorbancy was observed (less than 5%) during a period of 5 hours. The variation in molar

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TABLE I

COMPARISON AMONG GROWTH HORMONES FROM BEEF, HUMAN AND MONKEY PITUITARY GLANDS

	Beef	*Human*	*Monkey*
Assumed molecular weight (from amino acid composition)	46,000 (ref.[6])	28,000 (ref.[1])	28,000 (ref.[1])
Tyrosine residues per mole: Spectrophotometric method	12 ± 1	9 ± 1	10 ±
Paper-DNP method	11 (ref.[6])	5 (ref.[1])	7 (ref.[1])
Change in molar extinction at 295 mμ (per tyrosine residue)	2,400	2,250	2,400
Amide groups per mole	34	29	30
Calculated isoionic pH	7.0	5.1	5.8
Observed isoelectric pH	6.85 (ref.[2])	4.9 (ref.[9])	5.5 (ref.[1])
Mid-point of tyrosine ionization curve	11.2	10.7	10.75
pK_i of tyrosine (range, and most probable value)	10.45–10.6(10.45)	9.65–9.8(9.8)	10.2–10.3(10.25)
Electrostatic factor w (range, and most probable value)*	0.0317–0.0330(0.033)	0.053–0.055(0.055)	0.038–0.041(0.041)
Radius of anhydrous sphere	23.8 Å	20.2 Å	20.2 Å
Radius of equivalent electrostatic sphere Impenetrable by ions (ref.[3], eqn. 1)	30 Å	22 Å	26 Å
Permeable to ions (ref.[3], eqn. 15)	27 Å	21.5 Å	23.5 Å
Axial ratio of the equivalent hydrodynamic ellipsoid (prolate)	6.2	4.8	6.0**
Intrinsic viscosity (ml/g)	6.1	5.7	—

* Values of 0.034 for beef, 0.051 for human, and 0.040 for monkey are obtained when calculation is based upon the buffer value at the mid-point of the ionization curve (K. LINDERSTRØM-LANG, *Compt. rend. trav. lab. Carlsberg*, 15 (1924) No. 7).

** From sedimentation and diffusion data alone, assuming a hydration of 0.3 g/g protein.

extinction per tyrosine residue at 295 mμ, going from neutral to highly alkaline solutions, was of the order of 2,400, in complete agreement with similar data for free tyrosine (2,300), ribonuclease (2,630), and serum albumin (2,430)[7]. Accordingly, spectral changes could be used as a measure of the degree of ionization (α) of the tyrosine residues in the three hormones.

Calculation of the intrinsic pK (pK_i) and of the electrostatic factor (w) from the usual relationship[3] $\mathrm{pH} - \log(\alpha/1-\alpha) = \mathrm{p}K_i - 0.868wZ$ requires a knowledge of the net charge (Z) carried by the protein; in the absence of a complete titration curve, this quantity was estimated as follows. The isoionic pH was first computed from a separate determination of the amide content of the protein, from the known amino acid composition[1,6], and from currently accepted pK_i values for carboxylic, iminazole and α-amino groups[8]. This calculated isoionic pH, being practically identical with the experimental isoelectric pH[1,2,9], was taken as the origin of the Z axis. The variation of net charge in the pH range of from 9 to 12 was then ascribed to the ionization of tyrosine and ε-amino groups only. A series of plots of $(\mathrm{pH} - \log \alpha/1-\alpha)$ *versus* Z

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was accordingly made, the pK_i for ε-NH_2 being varied within the "normal" limits of 10.0 to 10.6 (*cf.*[8]). The most probable value for the pK_i of tyrosine was finally obtained by minimizing, with respect to the pK_i of ε-NH_2, the square of the deviations in those plots (Fig. 1).

The value $pK_i = 9.8$ for human growth hormone appears quite reasonable[7], in spite of the assumptions made during calculations for the purpose of simplification. In contrast, the high value obtained for the bovine hormone seemed to indicate that in this case some factor was actually depressing ionization. The tyrosyl–carboxylate ion hydrogen bonding discussed by SCHERAGA[10] indeed seems to occur in the bovine hormone, since the expected differential spectrum is obtained between pH 1 and pH 5 (Fig. 2); this spectrum is very similar to spectra recorded with insulin, ribonuclease,

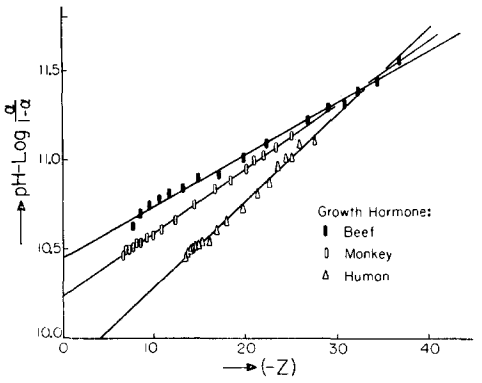

Fig. 1. Electrostatic interactions in growth hormones from three species. (Appropriate values for the pK_i of ε-amino groups are: 10.0 for the beef hormone, and 10.6 for the primate hormones.)

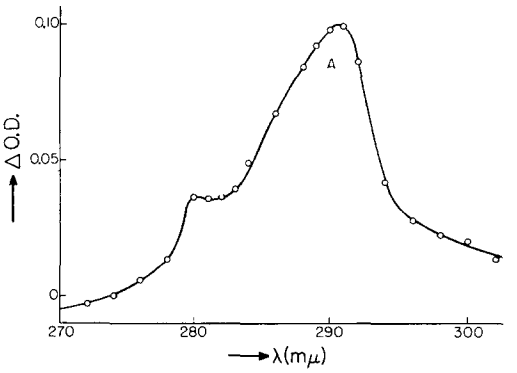

Fig. 2. Differential ultraviolet spectrum of bovine growth hormone, 1.9 m/ml, between pH 1.2 and pH 5.1. (Corrected for difference in solvent composition; readings were taken 1–2 hours after adjusting the pH, some time effect being observed.)

and lysozyme, although the main peak A is shifted by 3–4 mμ towards longer wave length. By comparison with ribonuclease, where the change in molar extinction at peak A has been evaluated[10], it would appear that 11–13 tyrosine residues are hydrogen-bonded in bovine growth hormone; this is entirely consistent with evidence, derived from the titration between pH 9 and 12, that all 12 tyrosines in this hormone behave in an identical manner. As expected, no significant differential spectrum was obtained with the human hormone (less than 2 hydrogen-bonded tyrosines out of 9). The differential spectrum of the monkey hormone will be worth investigating also, when more material becomes available, since its pK_i value seems to locate it in a position intermediate between the human and bovine compounds.

It is also of interest to compare, in connection with the three hormones, the radius of the anhydrous molecule, calculated from the values for molecular weight and partial specific volume, with the radius of the equivalent electrostatic sphere (Table I); the latter information can be derived from the electrostatic parameter w, assuming a suitable model[3]. The assumption that the growth-hormone molecules are impervious to ions from the solvent (equation 1 in ref.[3]) results in unexpectedly large radii for the equivalent electrostatic sphere; if hydration is considered responsible for the increases over the radius of the anhydrous molecule, values up to 0.8 g solvent/g protein are

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obtained. This model is clearly inadequate, as was often found to be the case with other proteins (see the discussion in ref.[3]). The assumption of a model which allows for permeability on the part of the molecule to ions*, on the other hand, leads to much more reasonable values for solvation factors (0.15 to 0.4 g/g protein) and for the radii of the equivalent particles.

When the human and monkey hormones are compared, it becomes obvious that the electrostatic parameter w does not necessarily parallel the size parameter measured by the molecular weight. Although this effect might possibly reflect some real difference in solvation between these two hormones, it could equally well arise from a different departure from spherical symmetry. In order to settle this point, separate determinations of intrinsic viscosity were carried out and the results were combined with available sedimentation and diffusion data in order to estimate the axial ratio of an hydrodynamic ellipsoid equivalent to the molecule[4]. It will be seen from Table I that the monkey hormone does indeed resemble the bovine hormone with respect to molecular asymmetry, although with respect to molecular weight it belongs to the same class as the human hormone; the relative values of the w parameter are thus readily explained on this basis. Finally, it seems worth mentioning that in the three proteins a striking parallelism is observed between molecular asymmetry and cystine content.

SUMMARY

The electrostatic and hydrodynamic properties of growth hormones isolated from bovine, monkey and human pituitaries have been investigated. It was noted that these three hormones differ with respect to the radii of their electrostatic spheres and the dissociation constants of their tyrosine phenolic groups. From the amide content determined herein and previous data on amino acid composition, isoionic points for bovine, monkey and human growth hormones have been computed to be 7.0, 5.1, and 5.8, respectively; these values are in fair agreement with the experimentally determined isoelectric points.

The differential ultraviolet spectrum of bovine growth hormone indicated the existence of a tyrosyl–carboxylate-ion hydrogen bonding. However, no significant differential spectrum was obtained with the human hormone.

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* Two slightly different models have been treated by TANFORD, one where the net charge of the protein is located entirely on the surface of the equivalent sphere (equations 14 and 17, in ref.[3]), and one where the net charge is distributed over the solvation shell (equation 15 in ref.[3]). The latter seemed preferable since the relationship between w and the radius is less dependent on the value assumed for the dielectric constant; this was taken as the dielectric constant of the solvent, although it is realized that a significant fraction of the interactions might actually be transmitted through the protein itself.