Histidine-rich Proteins As Molecular Markers of Epidermal Differentiation*

(Received for publication, October 21, 1977, and in revised form, March 13, 1978)

Richard D. Ball,† Glenn K. Walker,§ and Isadore A. Bornstein¶

From the Department of Biological Chemistry and the Cellular Chemistry Laboratory of the Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan 48109

Cells of the basal, spinous, and granular layers of the mammalian epidermis represent morphological stages in the differentiation of keratinocytes to form the outer cornified layer of the skin. In the newborn rat, concomitant with the transition of granular into cornified cells, there is a conversion of histidine-rich protein I (HRP), localized in keratinocytes of the epidermal granular layer, into HRP,. This transition from HRP, to HRP, provides a biochemical marker for this particular differentiative event.

HRP, and HRP, have similar amino acid compositions which are unusually high in polar amino acids (greater than 80 mol %) and histidine (approximately 7 mol %). As estimated from gel filtration studies in the presence of sodium dodecyl sulfate, HRP, exhibits a broad range of molecular weights from approximately 60,000 to greater than 100,000 whereas HRP, has an apparent molecular weight between 60,000 and 80,000. Peptide mapping of a tryptic digest of a mixture of HRP, labeled with [14C]histidine and HRP, labeled with [3H]histidine shows that of 18 peptides which are significantly labeled with [3H] and a similar number labeled with [14C], 11 of the [3H]-peptides chromatograph coincidentally with [14C]-peptides.

HRP, accounts for about 40% of the total nondialyzable [3H] in the epidermis after exposure in vivo for 1 h to [3H]histidine and about 30% after a similar exposure to an equally labeled mixture of the 20 common [3H]-amino-acids. HRP, constitutes only about 4% of the epidermal protein which is soluble in 8 M urea, 0.2 M Tris, pH 8.5, whereas HRP, is a major component and represents 35% of this extract. HRP, is present primarily in the cells of the lower cornified layers as demonstrated by autoradiography of histologically "pure" stratum corneum and isolation of the substance from such tissue preparations. HRP, is localized in the cells of the granular layers.

Available data support the hypothesis that the product of translation, HRP, is polymerized rapidly in the granular cells to a large polymer of heterogeneous size, HRP, which is degraded to a smaller molecule, HRP,, when the granular cell differentiates into a cornified cell, presumably, under genetic and/or epigenetic control. The conversion of HRP into HRP, can be used to study those regulatory processes which mediate epidermal keratinization, a specific example of eukaryotic differentiation.

Epidermal keratinocytes undergo a series of morphological and biochemical changes during their migration from the germinative basal layer to the outer cornified layer of the mammalian skin which results in the formation of stratified layers of cells (basal, spinous, granular, and cornified layers) representing stages of differentiation of the same cell type. Autoradiographic data have demonstrated that certain labeled amino acids, e.g. glycine and histidine, are incorporated initially in the upper, more differentiated layers of the epidermis, while other amino acids, e.g. leucine, phenylalanine, and methionine, localize initially in the lower layers of the epidermis. In support of this hypothesis, an unusual protein high in histidine (approximately 7%) and soluble in 8 M urea, pH 8.5, as well as in 0.1 M HClO₄ was isolated from the upper layers of the cutaneous epidermis of the newborn rat (5). The HRP fraction accounts for 35 to 50% of the total nondialyzable [3H]histidine incorporated into the epidermis 1 h after the subcutaneous injection of the tracer, and is localized in the epidermal layer immediately below the outer cornified layer, i.e. the granular layer (Fig. 1A) (7, 8).

The present paper concerns the properties of the above HRP, now designated HRP,, and reports the isolation and characterization of another HRP form, HRP,,, from the stratum corneum. HRP, is apparently derived from HRP,. Since HRP, is localized in the granular layer and HRP is localized in the cornified layers, the conversion of HRP to HRP, is assumed to be a molecular marker of the differentiation of a granular into a cornified keratinocyte. The conversion of HRP species provides a model in which to study the genetic/epigenetic "control processes" that regulate epidermal (eukaryotic) differentiation.

EXPERIMENTAL PROCEDURES

Methods

Preparation of Epidermis—Newborn rats (CFN strain, originally obtained from Carworth Farms, Inc., Rockland County, N. Y.) at 1 to 3 days of age, derived from a randomly inbred colony maintained in...
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**Isolation**

The isolation procedure for HRP, and HRP₂, which is based upon their unusual solubility properties and the scheme reported by Hoober and Bernstein (5), is shown in Fig. 2. The isolation procedure was applied to preparations of non-keratinized or keratinized cells (separately by scraping) or on the isolated epidermis directly. The precipitation of HRP₁ was carried out for 15 min at room temperature, rather than at 4°C overnight, as was done by earlier workers (5, 7, 12), since significant amounts of HRP₂ were also precipitated at 4°C and would have contaminated the preparation of HRP₁. This did not occur at room temperature (8).

**Determination of Protein and Radioactivity**—Protein determinations were performed with a Technicon autoanalyzer system using the method of Lowry et al. (13) as described by Mandl (14). Samples were prepared for measurement of protein concentration and radioactivity determination by adding 1 volume of 0.2 N NaOH, 1% ARW-7 (wetting agent-detergent from Technicon Corp.) and heating the sample in a boiling water bath for 5 min. Radioactivity determinations were performed by adding samples prepared in the above manner to about 10 ml of PCS (Phase-Combining System, Packard Instruments, Inc.) in minivials and counting in a Packard Tri-Carb scintillation counter. Efficiency of counting was 90% for ¹⁴C and 35% for ³²P.

**Gel Filtration Chromatography**—Gel filtration chromatography was performed using Sepharose 2B or 6B agarose gels (Pharmacia Fine Chemicals, Inc.). Data are reported in terms of $K_M$ and $V_{max}$ (15). All chromatographic columns (2.5 x 40 cm) were eluted at room temperature with a mixture of 0.1% SDS, 0.1 M NaCl, 0.001 M EDTA, and 0.01 M Tris, pH 8.5. When included in the above buffer, the concentration of 2-mercaptoethanol was 0.1%. Fractions collected were equal to approximately 2.5% of the total column volume. Void volumes were determined using dextran blue 2000 (Pharmacia Fine Chemicals, Inc.).

Samples were prepared for SDS-gel filtration chromatography by making the samples 1.0% in SDS, 0.1 M in NaCl, and 0.01 M in Tris, pH 8.5, heating in a boiling water bath for 5 min, stirring at room temperature for 3 h, and dialyzing overnight (2 x 50 volumes) at room temperature against the column-eluting buffer. When used in the above sample buffer, the concentration of 2-mercaptoethanol was 3%.

**Peptide Analysis**—Samples for peptide analysis were dissolved in 0.2 M ammonium carbonate, pH 8.8, containing 0.02 mg/ml of trypsin which had been treated with diphenylcarbamyl chloride to remove chymotryptic activity (Sigma Chemical Co., Catalogue No. T-1005). Samples (1 to 2 mg of protein/ml) were incubated at room temperature for approximately 16 h with the addition at 2 and 6 h of 0.2 ml of trypsin solution (1 mg/ml of trypsin in 0.001 N HCl) per 10 ml of digest medium. Samples to be co-chromatographed were digested as a mixture. In order to remove the salt, samples were then lyophilized for 12 h, resolubilized in a volume of 0.3 M acetic acid equal to the volume of the original digest, and again lyophilized for $12$ h before being dissolved in the initial column buffer and applied to the chromatographic column. Peptide analysis was carried out on a column (0.6 x 20 cm) of Aminex A-5 (Bio-Rad) by a modification of a Technicon peptide analysis procedure (16). The following elution...
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RESULTS

Gel Filtration Chromatography of HRP• Isolated from Nonkeratinized Cells—Fig. 3 shows a chromatogram on Sepharose 2B of HRP• isolated from nonkeratinized cells. HRP• was labeled in vivo by [14C]histidine for 24 h and [3H]histidine for 1 h. It had previously been shown that when radioactive histidine is used only histidine residues are labeled in HRP (19). Elution profiles of protein, [3H]histidine, and [14C]histidine were retarded on the gel under the above conditions, being eluted in the region of bovine serum albumin elution. No significant amount of 14C appeared in this fraction. Treatment of HRP• with 2-mercaptoethanol and inclusion of 2-mercaptoethanol in the eluting buffer did not alter the above elution profile. HRP• isolated directly from whole epidermis gave essentially the same elution profile of radioactivity and protein under the above conditions. When the leading edge of the elution profile from Sepharose 6B, as indicated in Fig. 4A, was collected, re-chromatographed on Sepharose 6B (Fig. 4B), and submitted to SDS-polyacrylamide gel electrophoresis, all the protein entered the gel and moved as a single sharp band (Fig. 5). On the second passage through Sepharose 6B, the trailing nonradioactive contaminant was absent and the specific activity of the protein increased.

Amino Acid Composition of HRP• and HRP•—Table I shows that the amino acid compositions of HRP• and HRP• are similar and unique but do exhibit minor variations. Arginine, lysine, glutamic acid, serine, threonine, histidine, aspartic acid, and glycine account for approximately 90 mol% of the residues present in both cases. Nonpolar amino acids are present in low amounts. As obtained from the whole epidermis by the same isolation procedure, both HRP• and HRP• have more alanine when isolated from whole epidermis. HRP• also has less alanine when isolated from whole epidermis.

Distribution of Radioactivity and Protein in the Urea-Gradient System—Table II shows the distribution of radioactivity and protein in the urea-gradient system. The elution profile and the specific activity of the radioactivity and protein were constant across the entire peak where significant measurements could be made. Approximately 15% of HRP• was retarded on Sepharose 6B when chromatographed under the above conditions and treatment of HRP• with mercaptoethanol and inclusion of 2-mercaptoethanol in the eluting buffer did not alter the elution profile (8). Chromatography of HRP• in a mixture of 8 M urea, 0.10 M NaCl, and 0.05 M Tris, pH 8.5, or 6 M guanidinium chloride, 0.10 M NaCl, and 0.10 M acetic acid, pH 4.5, did not result in any increase in protein or radioactivity being retarded on Sepharose 6B. Only a small portion (<20%) of HRP• entered the gel when the protein was submitted to electrophoresis on SDS-polyacrylamide gel (7%). The elution profiles of radioactivity and protein of HRP• isolated from whole epidermis were essentially the same as those obtained for HRP• isolated from the nonkeratinized cells.

Fig. 3. SDS-gel filtration chromatography of HRP• isolated from nonkeratinized cells. Animals were exposed to [3H]histidine for 24 h and [14C]histidine for 1 h in vivo. The column (2.5 x 40 cm) of Sepharose 2B was eluted with a mixture of 0.1% SDS, 0.10 M NaCl, 0.001 M EDTA, and 0.01 M Tris, pH 8.5, at room temperature. Vc = 70 ml. ●, protein; ▲, 14C; △, 3H.
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FIG. 4. SDS-gel filtration chromatography of HRPI isolated from keratinized cells. Animals were exposed to [3H]histidine for 24 h and [14C]histidine for 1 h in vivo. The column (2.5 x 40 cm) of Sepharose 6B was eluted with a mixture of 0.1% SDS, 0.10 M NaCl, 0.001 M EDTA, and 0.05 M Tris, pH 8.5, at room temperature. V0 = 75 ml. 

FIG. 5. SDS-polyacrylamide gel electrophoresis of HRPI. Sample consisted of combined fractions from the leading edge of the elution profile from Sepharose 6B as indicated in Fig. 4. Gels A, B, and C were loaded with approximately 10, 25, and 40 mg of protein, respectively. For technical details see "Methods."

TABLE I

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>HRPI (n=1)</th>
<th>HRPI (n=2)</th>
<th>Purified HRPI (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>0.9</td>
<td>0.7 ± 0.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>6.9</td>
<td>7.4 ± 0.6</td>
<td>13.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>14.0</td>
<td>12.9 ± 0.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>5.0</td>
<td>4.5 ± 0.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Threonine</td>
<td>6.7</td>
<td>6.2 ± 0.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Serine</td>
<td>15.1</td>
<td>16.4 ± 0.2</td>
<td>16.9</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>21.9</td>
<td>21.5 ± 0.2</td>
<td>20.8</td>
</tr>
<tr>
<td>Proline</td>
<td>10.8</td>
<td>11.3 ± 0.2</td>
<td>10.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>14.4</td>
<td>14.5 ± 0.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.0</td>
<td>4.5 ± 0.2</td>
<td>4.9</td>
</tr>
<tr>
<td>Cysteine</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Valine</td>
<td>0.7</td>
<td>0.3 ± 0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.8</td>
<td>1.0 ± 0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.5</td>
<td>0.5 ± 0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.0</td>
<td>0.3 ± 0.06</td>
<td>0.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

HRPI appears to represent a major portion of the de novo protein synthesis in the upper layers of the epidermis since when a tritiated amino acid mixture with approximately 5% of the total [3H] present in each of the 20 common amino acids was used as the tracer, HRPI incorporated about 25% of the incorporation of [3H] at approximately 1 h after the injection of the tracer (Fig. 6). A gradual decrease in this net incorporation followed which extended to 24 h, the longest time period used in this study. Relatively little [3H]histidine was incorporated into HRPI initially (0 to 8 h), but at 24 h administration of labeled histidine, HRPI accounted for 35 to 40% of the nondialyzable [3H] in the urea-soluble protein. The net incorporation of [3H] into HRPP at 24 h was roughly three times greater than the maximal net incorporation of [3H] into HRPI at any given time, suggesting the possibility that there is a flux of labeled histidine through HRPI into HRPP. While HRPI accounted for less than 10% of the nondialyzable HClO4-soluble [3H]histidine at 1 h after injection of the tracer, at 24 h it represented approximately 85%.

soluble Protein, HRPI, and HRPP after 1 H---Table II shows that approximately 86% of the total radioactivity present in the whole epidermis 1 h after subcutaneous injection of [3H]histidine was soluble in 8 M urea, 0.2 M Tris, pH 8.5, and was nondialyzable. Approximately 48% of this nondialyzable radioactivity was soluble in 0.11 N HClO4 at room temperature. Of the HClO4-soluble [3H], 92 and 7% were present in HRPI and HRPP, respectively. On the other hand, HRPP constituted about 4% and HRPP about 30% of the total protein in the urea-soluble fraction (Table II). HRPP, therefore, represented roughly 40% of the nondialyzable [3H] present in the epidermis 1 h after the injection of [3H]histidine, while HRPI was essentially unlabeled at this time. HRPI showed maximal net
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Table II
Distribution of protein and radioactivity (relative per cent) during the preparation of HRP₁ and HRPᵢ from whole epidermis

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein mg/g epidermis</th>
<th>Radioactivity mg/g epidermis - 10₀ * ^a</th>
<th>Relative specific activity * ^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea-insoluble fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondialyzed</td>
<td>94.3 ± 0.9 * ^c</td>
<td>41.2 ± 6.6</td>
<td>38.1 ± 6.8</td>
</tr>
<tr>
<td>Dialyzed</td>
<td>85.9 ± 2.0 * ^d</td>
<td>44.8 ± 7.2</td>
<td>5.0 ± 2.0</td>
</tr>
<tr>
<td>HClO₄-soluble fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRPᵢ fraction</td>
<td>4.1 ± 1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRPᵢ fraction</td>
<td>35.0 ± 4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea-insoluble fraction</td>
<td>4.5 ± 1.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ^a Determined as the sum of recovered ^3H in the nondialyzed, urea-soluble fraction and in the urea-insoluble fraction. The dialyzed urea-soluble fraction contained approximately 10⁸ cpm/mg of epidermis when the dose was 20 µCi/animal.

* ^b Based upon counts per min/mg of protein.

* ^c Standard deviation.

nondialyzable ^3H in the urea-soluble protein by 1 h after the subcutaneous injection of the tracer (8). HRPᵢ accounted for less than 1% of the nondialyzable labeled amino acid mixture incorporated into the urea-soluble protein at this time. This low level of incorporation is consistent with the low levels of ^3H found in HRPᵢ when only [^3H]histidine was used as the tracer.

Peptide Mapping of Tryptic Digests of [¹⁴C]Histidine-labeled HRP₁ and [¹⁴C]Histidine-labeled HRPᵢ—Comparison of the peptide maps derived from digestion of HRP₁ and HRPᵢ with trypsin should provide a measure of similarity of the primary structure of the two proteins. Fig. 7 shows the peptide map of a tryptic digest of a mixture of [¹⁴C]HRP₁ and [¹⁴C]HRPᵢ. HRP₁ was isolated from nonkeratinized cells after animals were exposed to [¹⁴C]histidine for 1 h. HRPᵢ was isolated from the keratinized cells 24 h after the rats had received [¹⁴C]histidine. Eighteen peptides of HRPᵢ were labeled significantly with ^3H and a similar number of peptides from HRP₁ were labeled with ^14C. Eleven ^3H-containing peptides from HRPᵢ co-chromatographed with ^14C-labeled peptides of HRP₁.

Localization of HRPᵢ in the Nonkeratinized Cells and HRPᵢ in the Stratum Corneum—Table III illustrates the distribution of protein and labeled histidine among the various fractions when HRP₁ and HRPᵢ were isolated from the nonkeratinized cells and the keratinized cells, respectively. These data approximate the values for HRP₁ and HRPᵢ obtained when the isolations were carried out on the whole epidermis (Table II). Table III shows that 70% of the protein, 86% of the [¹⁴C]histidine and 96% of the [¹⁴C]histidine in HRP₁ came from the nonkeratinized cells, while 93% of the [¹⁴C]histidine and 90% of the [¹⁴C]histidine in HRPᵢ were from the keratinized cells. These data support the view that HRP₁...
is primarily localized in the nonkeratinized cells while HRP II is essentially localized in the stratum corneum. To support this conclusion, autoradiography of the scraped stratum corneum labeled in vivo for 24 h with \[^{3}H\]histidine was done. As shown in Fig. 1C, there were large numbers of silver grains over the lower cornified layers, but few over the upper layers of this stratum. The stratum corneum contains no radioactivity that is demonstrable by autoradiographic techniques when the labeling period is less than 6 h (2). The appearance of autoradiographically detectable \[^{3}H\]histidine in the keratinized layers coincides temporally with the appearance of significant amounts of \[^{3}H\]histidine in the HRP II fraction (Fig. 6) and supports the localization of HRP II in the stratum corneum.

**DISCUSSION**

HRP I and HRP II represent major products of epidermal differentiation, since together they account for 40 to 45% of the epidermal protein which is soluble in 8 M urea, 0.9 M \(\text{Tris}\), pH 8.5, and represent approximately 12.5% of the wet weight of the tissue. As judged by the relatively high degree of labeling of HRP I by a tritiated acid mixture in vivo, synthesis of HRP I represents a major portion of the total protein synthesis in the cells of the upper layers of the epidermis. Based on its elution profile on Sepharose 2B in SDS-containing buffers (Fig. 3), HRP I appears to exist in a range of molecular weights estimated to extend from approximately 60,000 to as high as 10^6. The high molecular weight of HRP I could not be reduced by common protein denaturants, e.g. 8 M urea, 6 M guanidinium chloride, or by reduction with 2-mercaptoethanol in the presence of SDS. HRP I and HRP II are different entities since when isolated from the same epidermis, they show different specific radioactivities (Table II) and in experiments carried out over a period of 24 h of exposure to labeled histidine, the initially highly labeled HRP I decreased in specific radioactivity while HRP II became increasingly radioactive (Fig. 6). At each time point in these experiments, the same amounts of HRP I and HRP II were isolated per g of epidermis.

Since the molecular weights of single, unaltered polypeptides of ribosomal origin are rarely larger than 125,000 to 150,000, the above results suggest that HRP I represents a polymer containing a form of HRP (HRP II) which is encoded in mRNA and is linked by covalent, non-disulfide bonds in the large molecule. No HRP II was observed in the present investigation in vivo. However, in previous studies in vitro (24), in a puromycin-sensitive reaction, labeled histidine was incorporated into a small molecule that was soluble in 0.1 M HClO₄ and remained soluble at pH 4.5. The small molecule was then converted in the presence of puromycin to a larger molecule which was soluble in HClO₄ but was precipitated at pH 4.5.

The working hypothesis that HRP I is a polymer of heterogenous size consisting of various numbers of subunits (HRP I) is based upon (a) the constant specific activity in counts per min/mg of protein across the peak when HRP I labeled with radioactive histidine was chromatographed on Sepharose 2B (Fig. 3) and (b) the similarity in the unique amino acid composition of HRP I at various stages during its purification (8) and the presence in the newborn rat epidermis of a protein oligomeric series with molecular weights from 10^6 to 6 \times 10^4 which has essentially the same amino acid composition as HRP I.

Previous studies (19) have indicated the absence of carbohydrate. The nature of the putative linkages in HRP I is not known, although it has been reported that HRP I contains lysine residues with the e-amino group blocked in an undetermined manner (25). Since the granular layer of the epidermis is known to contain large amounts of transglutaminase, the enzyme responsible for forming \(\gamma\)-glutamyl-e-lysine linkages from polypeptide-bound glutamine and lysine (26-28), inter-chain \(\gamma\)-glutamyl-e-lysine cross-links may play a role in forming the HRP I polymer and be broken in the conversion of HRP II to HRP I. Work in progress to determine the composition of the tryptic peptides should clarify the nature of the linkages.

The absence of disulfide linkages in HRP I is supported by the results of earlier studies (a) in which the amino acid analysis of HR P I included a procedure to oxidize cysteine to cysteic acid but no cysteic acid was observed and (b) in which HRP II was isolated from animals which had been exposed to \({^3}S\) cysteine for 1 h in vivo but no \({^{35}}S\) was found in the HClO₄-soluble, pH 4.5-insoluble tissue fraction (18). The absence of cysteine has been confirmed by other investigators (29-32).

The present results with respect to the molecular weight of HRP I, conflict with those of previous workers (5, 7, 12) who observed molecular weights ranging from 30,000 to 390,000. Lower molecular weights for HRP I were obtained in the present investigation only when the protein was exposed to sodium carbonate buffers, e.g. 0.05 M Na₂CO₃, for 12 to 24 h at room temperature (8) as was done in the original procedure (5). This step was omitted in the present procedure. The basis for this phenomenon is not understood. It did not occur at room temperature in 0.1 M acetic acid or 0.05 M NaHCO₃ with or without 0.1% SDS (8).

Attempts to isolate HRP I from the liver of newborn rats by extraction with urea followed by exposure to 0.1 M HClO₄ and gel filtration chromatography in SDS have been unsuccessful (8, 32).

The epidermal preparations from which HRP I was isolated in this study included upper spinous, granular, and cornified keratinocytes. However, isolation of HRP I from epidermal preparations which contained only granular and cornified cells did not result in a significant decrease in the yield of HRP I (8, 32). The epidermal preparations used in this study included upper spinous, granular, and cornified keratinocytes.

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temporarily with the initial detection of \(^1\)H in the stratum corneum by autoradiographic techniques (2); and (e) HRP\(_{II}\) is localized in older, more differentiated keratinocytes than is HRP\(_I\). Proof that HRP\(_I\) arises from HRP\(_{II}\) must await the results of a future study of the process.

Recently, Dale reported (29) the isolation from the stratum corneum of a basic protein having a molecular weight of 50,000 and an amino acid composition similar to that of HRP\(_I\) and HRP\(_{II}\). This basic protein has a high level of histidine and no sulfur and might be formed from HRP\(_{II}\). The protein isolated by Dale may be the unlabeled protein which followed HRP\(_{II}\) in the elution from Sepharose 6F (Fig. 4A). This nonradioactive contaminant of HRP\(_I\) probably had a composition similar to HRP\(_{II}\) since the material submitted to chromatography on Sepharose 6F had an amino acid composition only slightly different from that of the purified HRP\(_I\); although the contaminant represented a significant fraction of the total protein put on the column. If this contaminant (HRP\(_{II}^?\)) is indeed derived from HRP\(_{II}\), it should become labeled at some time subsequent to 24 h after exposure to radioactive histidine in vivo.

Histidine-rich proteins have also been isolated by Freedberg and colleagues from the epidermis of newborn rat by extraction with deoxycholate followed by molecular sieve chromatography (30, 31) and by Balmain (34) from the epidermis of the adult mouse after treatment of the animal with phorbol esters.

A model for HRP biosynthesis, based on the above data, is shown in Fig. 8. HRP\(_I\), the putative "ribosomal" form of HRP (24), is synthesized in the granular layer of the epidermis and polymerized to form HRP\(_{II}\). Concomitant with the conversion of granular keratinocites to cornified keratinocytes, HRP\(_I\) is converted to HRP\(_{II}\). The conversion of HRP\(_I\) to HRP\(_{II}\) is possibly the result of a specific type of proteolytic cleavage.

The epidermis offers a system in which it is technically easy to isolate subpopulations of keratinocytes which have, and have not, undergone one major morphological differentiative step. These subpopulations of keratinocytes present the advantages that they can be isolated from the same animal, therefore serving as their own controls, and that most experiments can be run in vivo without physiologic perturbation of the organ. The above features are in contrast to those of other systems currently used to study eukaryotic differentiation, e.g. the erythroid system in which there are difficulties in in situ, and most experiments must be done in vitro (35). In addition, the biosynthetic system for HRP is apparently perturbed in several disorders of epidermal proliferation and differentiation, e.g. psoriasis, ichthyosis vulgaris, and other ichthyoses (3, 36) and appears to be affected when the normal rigidity which the cellular membrane achieves as the granular cell cornifies (42). The availability of keratinocyte cultures, which differentiate in vitro to form tonofilaments, keratohyalin (47), and cornified envelopes (48) should be useful for clarifying the relationships between the various biochemical and morphological steps in epidermal differentiation.

Acknowledgments—Our special thanks are extended to Doctor William P. Winter and Dorothy Sweet for assistance in performing the amino acid analyses and to Mark Krauss for assistance with the electrophoretic analyses.

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Histidine-rich proteins as molecular markers of epidermal differentiation.
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