Attention is next paid to the two bovine thyrotropins, as well as the various gonadotropins and luteinizing hormones. These proteins are related through various common sequences[77,78] and are fitted to placental lactogen at positions 61–63 and 68–70. It is recognized that these compounds have a larger number of differences with the growth hormones and are probably only distantly related at this time. The placement of BLUT-β and BTTβ with respect to BTTα is based on common residues found at positions 112–117, yet can stand improvement in the initial N-terminal section. On turning to the various proinsulins, which includes nerve growth factor and probably parathyroid hormone and β-corticotropin, alignments are easily found, especially with the cysteines located at positions 37, 62, and 89–90. Angler fish insulin is included now since it contains both an additional residue and various changes in the N-terminus of the β-chain. The homology between these groups disappears rather rapidly after the 44–47 area, possibly as a consequence of the initiation of the connecting peptide sequence found in the insulins, or of the need to introduce gap areas in order to maintain the alignments. The recent detection of a proparathyroid hormone is of much interest[79,80], since it possesses 15–20 additional residues, which presumably must lie to the left and right of the present parathyroid sequence and should support the present assignments. The inclusion of porcine pepsinogen, bovine pepsinogen, and bovine rennin at this time is based on their resemblance to the initial sections of glucagon and secretin, as well as similar regions in the various growth hormones. Finally, the three calcitonins appear to be generally related to both OLAC and the proinsulins.

The compounds discussed here must have arisen from a series of ancestral homotypes that evolved by the general mechanism of gene duplication. The original parent was probably present in the digestive tract and in time was modified to fit various roles through changes occurring in the gut and accessory regions. It is likely that the commonality in the first 30 residues for many of these compounds may be a result of the conservation of binding to a specific receptor site, followed by the activation of the adenylate cyclase system, or by the release of inorganic ions[81], transmitter substances, and by serving at the level of transcription[82]. The empirical relationships established here clearly show that the various gastric hormones constitute a homologous block of compounds, which served in time to develop the insulins, and, ultimately the growth and luteinizing hormones. On these grounds, one can find reduplicated sections beginning at positions 51, 91, and 131, suggestive of internal gene doubling patterns. Computer techniques that have been proposed for the testing of homology[83–86] will be used in the near future in an attempt to confirm these assignments. Even now indications exist that thrombin is a member of this large family. In summary, attention is called to the old folk proverb that maintains, 'the way to a man's heart is through his stomach'. This statement is undoubtedly true and probably should be modified to include other organs as well[87].

Résumé. La calcitonine, la cholécystokinine, la motilin, la pancreozymine, le peptide inhibiteur gastrique, le peptide vasoactif, la gastrine, le glucagon, et la secretine sont comparables par leur composition en amino acides a l'hormone de croissance, à l'hormone luteinigène, la gonadotropine, au facteur de croissance nerveux, à l'hormone parathyroidienne, au lactogène du placenta, à la proinsuline, à la rénine et à la thyrotropine. Ces peptides proviennent probablement d'un génotype dont l'origine se trouve dans le système digestif.

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PRO EXPERIMENTIS

The Purification of Peptic Antibody Fragments from Rabbit Immunoglobulin G

Univalent antibody fragments of the type Fab' are obtained by sequential treatment of immunoglobulins with pepsin and thiol compounds[1]. However, experience in our laboratory has shown that published methods do not produce physically homogeneous proteins. Therefore, we have devised a scheme that effectively uses gel permeation chromatography for purification[2].

To 1 volume of rabbit serum was added saturated $(NH_4)_2SO_4$ to 40% saturation[3]. The precipitated globulins were recovered by centrifugation (2,500 *g* for 10 min), dissolved in 1 volume water, and twice reprecipitated as before. The final precipitate was taken up in $^1/_6$ volume water and extensively dialyzed against 15 m*M* potassium phosphate, pH 8.0. Insoluble material was removed by centrifugation (8,000 *g* for 10 min). The supernatant was chromatographed on diethylaminoethyl (DEAE)-cellulose[4] using the same buffer. The immunoglobulin G (IgG) peak emerging in the void volume was dialyzed against 0.1 *M* sodium acetate, pH 4.5, and the contents of the dialysis bag were concentrated with Ficoll[5] to 15 mg/ml ($A_{280\ nm}^{mg/ml}$ 1.29).

To 500–750 mg IgG was added pepsin[6] at a pepsin-to-globulin ratio of 1:50. The mixture was gently agitated at 37 °C for 8 h. The reaction was stopped by raising the pH of the solution to 7.6 and by chilling. Insoluble material was removed by centrifugation (24,000 *g* for 10 min). After concentration the supernatant was applied to a column (2.8 × 45 cm) of Sephadex G-150 superfine[5].

Elution with 0.05 M potassium phosphate, pH 7.2 gave the profile shown in Figure 1. The fractions containing the $F(ab')_2$ fragments (determined to have $s_{20,w}$ 5.1 S; literature value, 4.8–5.0 S[1]) were dialyzed against 0.1 M sodium acetate, pH 5.0 and concentrated to 25 mg/ml ($A^{mg/ml}_{280\ nm}$, 1.48).

The $F(ab')_2$ fragments were reduced with 0.01 M mercaptoethylamine hydrochloride at 37 °C for 90 min under nitrogen. Iodoacetamide was added to give a 20% molar excess over mercaptoethylamine, and carboxamidomethylation (CAM) was allowed to proceed at 20 °C for 15 min. This was followed by blowing oxygen over the surface of the solution at 20 °C for 60 min. The reaction mixture was then chromatographed on the same column of Sephadex G-150 superfine as before. The protein peak corresponding to the crude CAM-Fab′ fragments (Figure 1) was further purified on a colum (2.5 × 90 cm) of Sephadex G-100 with 0.05 M potassium phosphate, pH 7.2 being used as the eluent. The first protein peak emerging from the column was discarded, and the major fraction (Figure 1, inset) was collected as the pure CAM-Fab′ fragments ($A^{mg/ml}_{280\ nm}$, 1.48). Starting from 250 mg IgG (the amount obtainable from 1 rabbit), the yield was approximately 20 mg.

The purified CAM-Fab′ fragments were judged to be physically homogeneous by the criterion of single symmetrical peaks obtained on Sephadex G-100 chromatography (Figure 1, inset) and on analytical ultracentrifugation (Figure 2)[7]. Immunoelectrophoresis and double gel diffusion of the parent IgG and $F(ab')_2$ preparations with the use of goat antiserum to rabbit whole serum[8] revealed a single precipitin line indicating absence of any significant contamination by other serum proteins and gave evidence of an antigenetic identity between the two preparations. On the other hand, the antiserum did not react with the final CAM-Fab′ fragments indicating a loss of antigenicity with respect to the particular antiserum used.

In conclusion, the reported method yields, by way of the intermediate $F(ab')_2$ fragments, S-carboxamidomethylated Fab′ fragments from rabbit IgG that seem to be physically homogeneous by gel filtration and analytical ultracentrifugation. No contamination by other serum proteins can be detected by immunoelectrophoresis and double gel diffusion[9].

Zusammenfassung. Es wird eine Methode beschrieben zur stufenweisen Isolierung von peptischen Antikörperfragmenten ($F(ab')_2$ und Fab′) aus Kaninchen-Immunglobulin G. In jeder Stufe der Präparation erfolgt die Reinigung mittels Gelpermeationschromatographie. Das Kriterium eines symmetrischen Piks bei analytischer Ultrazentrifugation und Gelfiltration lässt das gereinigte Schlusspräparat physikalisch homogen erscheinen. Verunreinigung durch andere Serumproteine können aufgrund der Immunelektrophorese und der doppelten Geldiffusion ausgeschlossen werden.

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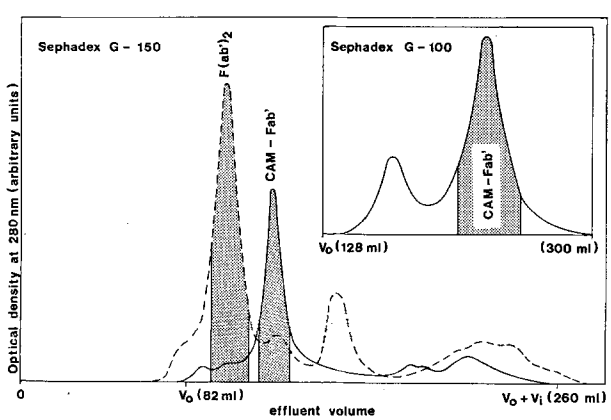

Fig. 1. Elution profiles observed on Sephadex G-150 in the isolation of $F(ab')_2$ fragments (---) and of the derived CAM-Fab′ fragments (—) from rabbit IgG. The stippled areas under the curves represent the fractions collected. The inset shows the gel filtration pattern as obtained on rechromatography of the crude CAM-Fab′ fragments on Sephadex G-100.

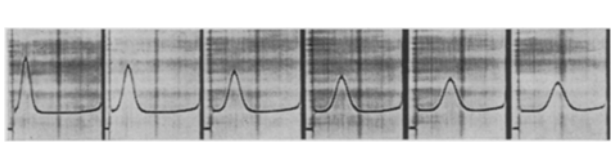

Fig. 2. Analytical ultracentrifugation profile of the purified CAM-Fab′ fragments. The run was performed in a Beckman model E ultracentrifuge equipped with schlieren optics (schlieren angle, 65°). An aluminum standard cell (12 mm, 4°) was used with an AN-D rotor. The protein concentration was 10 mg/ml. Solvent, 0.05 M potassium phosphate, pH 7.2. Temperature, 20.0 °C. Rotor speed, 56,100 rpm. Time interval, 16 min. Evaluation of the plot gave $s_{20,w}$ 3.8 S, which was in satisfactory agreement with the reported value of 3.5 S[1].

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[2] We are aware of the fact that most research groups utilizing antibody fragments have developed similar purification methods. Nevertheless, we feel compelled to publish the present procedure in view of our experience that detailed accounts of the various techniques are not readily available in the scientific literature, which also lacks a thorough characterization of the isolated products.

[3] All operations were carried out at 2–4 °C unless otherwise stated.

[4] Analytical grade, Serva Entwicklungslabor, Heidelberg, Germany; 0.5 g DEAE-cellulose was used per ml of original serum volume.

[5] Pharmacia, Uppsala, Sweden.

[6] From hog stomach mucosa, Boehringer GmbH, Mannheim, Germany.

[7] Attempts to further assess the purity of the preparation by polyacrylamide gel electrophoresis in buffers containing sodium dodecyl sulphate were unsuccessful since complex patterns resulted from extensive aggregation and recombination of the fragments.

[8] Miles Seravac.

[9] We thank Dr. R. von Fellenberg for his help with the immunochemical analysis. The critical reading of the manuscript by Dr. L. K. Wynston is gratefully acknowledged.