partir des drosophiles «sepia»[5], de la façon suivante: extraction par macération dans l'éthanol à 70%, élimination de l'alcool, centrifugation du résidu, adsorption des substances fluorescentes sur Florisil 60–100 mesh à pH 4, élution à l'acétone à 30%. Séparation des sépiaptérines de la riboflavine sur QAE-Sephadex A-25. Isolement des premières sur papier Whatman No 1 dans le butanol-acide acétique-eau (4/1/1), puis l'eau. Les autres substances sont des préparations commerciales: ptérine et isoxanthoptérine (Fluka), bioptérine (Smith, Kline & French) et riboflavine (Calbiochem). L'«extrait brut» est l'extrait précédent avant son passage sur Florisil.

Résultats. Les meilleurs séparations ont été obtenues à des pH voisins de 4,5–5,0; des valeurs plus faibles diminuent la résolution des ptérines entre elles sans affecter notablement celle de la riboflavine et des sépiaptérines; des valeurs plus élevées ont un effet contraire. La figure 1 représente un fractionnement sur une colonne de 2 cm de diamètre et de 70 cm de hauteur, à pH 4,7. Le premier pic (A) correspond à la riboflavine, le second (B) aux sépiaptérines qui ne sont pas séparées, les suivants aux trois autres ptérines.

Le fractionnement de l'«extrait brut» effectué à pH 4,3 sur une colonne de 3,8 cm de diamètre et de 70 cm de hauteur, a donné des résultats analogues aux précédents (Figure 2). Il en a été de même avec des colonnes plus étroites (0,8 cm).

Pour divers diamètres de colonnes, la séparation sépiaptérines-riboflavine se fait dès les 50 premiers centimètres, mais 70 cm sont nécessaires pour qu'elle soit complète. Les pertes subies pendant le fractionnement sont différentes suivant les substances: les rendements ont été respectivement de 66 et 89% pour la sépiaptérine et la riboflavine, dans le cas de deux colonnes (diamètre 1,2 cm, hauteur 70 cm), chacune chargée avec 30 µg de l'une des deux substances. Les résultats de ces fractionnements ont été comparés à ceux donnés par le QAE-Sephadex A-50 de degré de réticulation plus faible et le DEAE-Sephadex A-25 échangeur d'anions faibles. Dans les deux cas, les résolutions de tous les pics sont été bien moins prononcées.

Conclusion. Le QAE-Sephadex se comporte comme un tamis moléculaire vis-à-vis de la riboflavine et des sépiaptérines dont les charges sont voisines, mais un échangeur d'ions vis-à-vis de l'ensemble des ptérines. Il possède un pouvoir de résolution prononcé. Les sépiaptérines, sans être séparées entre elles, sont isolées de la riboflavine comme de plusieurs autres ptérines de charges voisines (la migration des drosoptérines – labiles – se fait avec destruction dans la colonne). Il convient aussi bien pour de faibles que de fortes quantités de substances et complète les données fournies par la poudre de cellulose.

Summary. Sepiapterins are easily separated from riboflavin and other weakly charged pterins, on QAE-Sephadex A-25.

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Preparation of Carbohydrate Modified Proteins

Mono- and oligosaccharides may be attached to protein molecules by diazo-coupling of the *p*-aminophenol glycosides, mainly by reaction with the tyrosine residues occuring in the proteins[1]. However this type of linkage has not yet been found in nature, and the presence of aromatic rings near the carbohydrate-protein bond may influence the immunological properties of the product.

We can now describe a method which enables glycosylamines to be attached to the carbodiimide activated carboxyl groups of the proteins[2]. In this series of experiments we used egg white lysozyme (Sigma, 3 × crystallized) as protein component, and α-D-galactosylamine NH_3-complex[3], lactosylamine[4] as well as D-glucosamine as sugar derivatives.

The reaction mixture contained 10 mg of lysozyme and 1.0 mmol of glycosylamine or glucosamine, dissolved in 8 *M* aqueous urea (1 ml) or 5 *M* guanidine HCl (1 ml). 20 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (OTT chemical Co.) dissolved in 0.5 ml urea (8 *M*) or guanidine HCl (5 *M*) was then added, at room temperature to initiate the reaction. The pH was maintained at 4.75 by automatic titration in a Radiometer pH-stat with 4.0 *M* HCl. 20 min after the end of the acid uptake (35-40 min) the solution was dialyzed against 3 changes of 0.001 *M*

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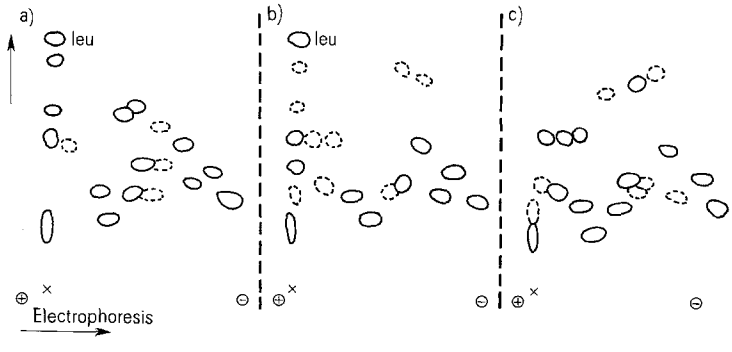

Fig. 1. Peptide map of a trypsin digest of reduced, carboxymethylated lysozyme and its glycosylated derivatives on silica thin layers. Peptides detected by ninhydrine. Electrophoretic step: pyridine-acetic acid-water 1:10:89, 2 h, 300 V; chromatography: isoamylalcohol-pyridine-water 7:8:6. Leu = leucine. a) lysozyme. b) galactosylamine. c) lactosylamine derivative.

HCl, finally against distilled water, and the product was lyophilized.

The hexose content of the lysozyme-galactosylamine reaction product determined by the orcinol method was 2.0% (1.6 galactose/mol protein) in urea and 2.8% (2.2 galactose/mol protein) in guanidine HCl solution. The lactosylamine derivative obtained in guanidine HCl solution contained 8.8% of hexose (3,6 lactose/mol protein). The coupling of D-glucosamine under the same conditions yielded a product containing 1.5% (1.2 ml glucosamine/mol protein) glucosamine, as determined by the Morgan-Elson method.

The presence of galactose, galactose plus glucose, or glucosamine respectively was demonstrated in the acid hydrolysates of the products by thin layer chromatography[5].

The electrophoretic mobility of the galactosylamine and of the glucosamine derivatives in acrylamid gels in the presence of sodium dodecyl sulfate[6] was essentially the same as of the untreated lysozyme. The lactosylamine derivative migrated somewhat slower, as could be expected from the gain in molecular weight due to the carbohydrate residues.

Peptide maps of the carboxymethylated[7] products were performed on silica thin layer[8] (Figure 1) after trypsin digestion[9]. Sugar-containing peptides were demonstrated on thin layer electrophoregramms detected successively by ninhydrine and by orcinol-sulfuric acid reagents[8] (Figure 2).

The fingerprints of both the lactosyl- and the galactosylamine derivatives differ significantly from that of the lysozyme. The absence of the C-terminal leucine on the peptide map of the lactosylated products indicates that the C-terminal carboxyl was quantitatively modified by the lactosylamine. The presence of 3.6 mol of lactose in one mol of protein, as well as the presence of several sugar-containing peptides in the trypsin digest, suggest that the major part of the lactosylamine must be bound to the free carboxyl groups of glutamic and/or aspartic acid residues (2 glutamic and 7 aspartic acid residues were found in lysozyme by CANFIELD and ANFINSEN[9].

The orcinol reaction of the trypric peptides of the galactosylated product on the electrophoregrams of silica thin layers was much weaker than those of the lactosylamine derivatives. The leucine spot on the peptide map of the galactosylderivative was almost as accentuated as on the corresponding peptide maps of the unchanged lysozyme. This suggests that none of the available carboxyl groups of this product is glycosylated quantitatively under the conditions used.

The presence of the sugars in a non-dialysable, electrophoretically homogenous protein derivative, as well as the presence of sugar-containing peptides in its tryptic digest, indicate that this simple method permits the introduction of well defined oligo- and monosaccharides into protein molecules under mild conditions by means of a naturally occurring glycosylamine bond.

Résumé. Mono- et oligosaccharides ont été attachés au lysozyme par une liaison glycosylaminique, en faisant réagir les protéines activées sur les groupements carboxyliques par carbodiimides, avec des glycosylamines.

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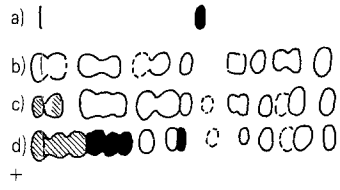

Fig. 2. Electrophoregramm of the tryptic peptides on silica thin layer. Buffer: pyridine-acetic acid-water 1:10:89, 2 h, 300 V. ○, ninhydrine positive sports; ●, ninhydrine and orcinol positive spots, a) glycosylgalactosylhydroxylysine; b) lysozyme; c) galactosylamine-; d) lactosylamine derivative.

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A Microlathe for Constructing Miniature Multibarrel Micropipettes for Iontophoretic Drug Application

Various techniques for the construction of multibarrel micropipettes for the micro-iontophoretic application of drugs to neurones have been published. CURTIS[1] has described a large multibarrel electrode (Figure 1) which must be handmade by an experienced glassworker, a limitation which together with the large drug volume required, reduces its utility. HERZ et al. (in KRNJEVIC[2]) have described a technique using cemented glass tubes which are twisted together just prior to pulling. Unfortunately, while this technique produces excellent small-sized electrodes, considerable time must be devoted to acquiring the technique; the electrodes tend to be very variable in their properties and dimensions and the tip length can seldom be longer than 5 mm.

The microlathe described here permits the construction of electrodes having 3 or more barrels with reproducible characteristics and with small (1.5–2 mm) diameter tip extensions of up to 2 cm or more which permits their use in investigating deep brain nuclei. This microlathe, the design of which is based on the conventional glassworkers lathe, allows simultaneous rotation, heating, fusing, and drawing apart of the softened glass electrode blank. The essentials of the lathe are evident from Figure 1.

An aluminium optical bench (B) forms the bed of the lathe upon which a platform (P) can be moved by means of a rack and pinion (R)[3]. It is imperative that the pair of gears on the head and tailstock should have the same

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