d. *Val·Ala·Try·Arg*. Par hydrolyse totale acide, (Val, Ala, Arg) ont été obtenus en quantités équimoléculaires. La présence de Try a été décelée spécifiquement sur le peptide entier[2]; par la méthode d'EDMAN[4], il a été possible de détacher successivement: Val, Ala et Try. L'arginine libre a été retrouvée à la fin de l'opération.

Scission de la leucine C-terminale. Il est intéressant de noter que, après traitement du lysozyme par la trypsine dans les conditions réalisées ici, on trouve, comme acides aminés à l'état libre, uniquement de la lysine, de l'arginine et de la leucine en quantités inférieures à une molécule pour une molécule de lysozyme. La lysine ainsi libérée est évidemment la lysine N-terminale; l'arginine ne peut provenir que d'une liaison Lys·Arg ou Arg·Arg; quant à la leucine, elle ne peut représenter que la leucine C-terminale[8,9]. Il en résulte que l'acide aminé précédant immédiatement la leucine est soit la lysine, soit l'arginine.

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[1] A. P. RYLE, F. SANGER, L. F. SMITH ET R. KITAI, *Biochem. J.*, 60 (1955) 541.
[2] J. TABONE, D. ROBERT, S. THOMASSEY ET N. MAMOUNAS, *Bull. soc. chim. biol.*, 32 (1950) 529.
[3] F. SANGER, *Biochem. J.*, 39 (1945) 511.
[4] P. EDMAN, *Acta Chem. Scand.*, 4 (1950) 283.
[5] R. ACHER, C. FROMAGEOT ET M. JUTISZ, *Biochim. Biophys. Acta*, 5 (1950) 81.
[6] A. R. THOMPSON, *Biochem. J.*, 61 (1955) 253.
[7] R. ACHER, J. CHAUVET, C. CROCKER, U. R. LAURILA, J. THAUREAUX ET C. FROMAGEOT, *Bull. soc. chim. biol.*, 36 (1954) 167.
[8] J. I. HARRIS, *J. Am. Chem. Soc.*, 74 (1952) 2944.
[9] A. R. THOMPSON, *Nature*, 169 (1952) 495.

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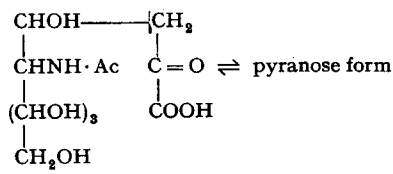
The linkage of sialic acid in mucoprotein

It has been established that sialic acid[1] and N-acetyl neuraminic acid[2] respectively are components of many mucoproteins and that 2-carboxypyrrole isolated[3] from the alkali hydrolysate of these mucoproteins is derived from neuraminic acid[4]. The structure shown below was recently assigned[5] to N-acetyl neuraminic acid (I) on the basis of the analytical data available and is supported



by the isolation of 2-carboxypyrrole from a Knorr-type condensation of D-glucosamine with pyruvic acid. The characteristic features of I, (i) the aldol type of linkage between N-acetyl hexosamine and pyruvic acid providing favourable conditions for the production of 2-carboxypyrrole upon alkali treatment and (ii) the presence of an α-keto acid grouping yielding readily to decarboxylation by mineral acid with the formation of a substituted 2-deoxy aldose, account for most of its chemical properties. Sialic acid has an additional O-acetyl group which is easily split off[6] attached to one of the C atoms 4, 6, 7, 8 or 9.

Human urine mucoprotein[2] (UM) and bovine submaxillary gland mucoprotein[7] (BSM) are known to contain 4% I and 17% sialic acid respectively. When acted upon by the influenza virus enzyme or by the receptor destroying enzyme (RDE) of vibrio cholerae, these mucoproteins release a dialysable substance engaging in the same chemical reactions as do the acetylated neuraminic acids[8]. The substance liberated from UM was identified as I[2]. These observations would suggest that mono- and diacetyl neuraminic acid respectively occupy terminal positions in the mucoprotein molecule. For UM it is known that neuraminic acid is located in the carbohydrate-prosthetic group[9].

Both neuraminic acids have strong reducing power in analogy to that of 2-ketogluconic acid.

It was therefore surprising to find that a 2% solution of BSM did not reduce Benedict's reagent. When, however, NaOH was substituted for Na_2CO_3, as in Fehling's solution, reduction took place. Thus 90 mg BSM, when submitted to the quantitative procedure of Fehling-Lehmann-Maquenne-Schoorl[10] (the temperature within the tube kept for 2 min at 100°), had a reducing power corresponding to 3.5 mg glucose, which is equivalent[1] to 5.8 mg sialic acid. This finding indicated the masking of the reducing group of sialic acid within the BSM molecule by an alkali-sensitive glycosidic link. To obtain further information the following experiments using mild acid as hydrolysing agent were carried out:

(a) *Identification of sialic acid*: 90 mg BSM were dissolved in 3.5 ml water, heated at 80° for 1 h at pH 1.0 (adjusted with *N* H_2SO_4), cooled and centrifuged (10,000 × *g*, 30 min, 4°). The clear supernatant, after removal of H_2SO_4 as barium salt, reduced Benedict's reagent, gave Bial's orcinol reaction with violet colour, a direct Ehrlich reaction on heating (purple; λ max. = 565 mμ) and a diphenylamine reaction (blue; λ max. = 532 mμ), as described for sialic acid[7]. When the supernatant was heated with $Ba(OH)_2$ (0.5 *N* final conc., 7 h, 100°), cooled and acidified, a substance was extractible with ether which chromatographically and spectrophotometrically (λ max. = 256 mμ, phosphate buffer, pH 7.0) proved to be identical with 2-carboxypyrrole. In addition to sialic acid or I the supernatant contained 20% of the residual mucoprotein as determined by weight. Chromatography of the concentrated supernatant using Whatman No. 1 paper and butanol/pyridine/H_2O (6:4:3) as solvent did not reveal the presence of any of the component sugars of BSM though by the technique applied a hydrolysis of even 10% of the polysaccharide of BSM would have been detected[11].

(b) *Quantitation of liberated sialic acid*: 90 mg BSM treated as under (a). The reducing power (Fehling-Lehmann-Maquenne-Schoorl) of the supernatant, after neutralization with *N* NaOH, corresponded to 7.30 ± 0.15 mg glucose, equivalent to an average of 11.9 mg sialic acid, *i.e.* about 78% of the sialic acid content of the original BSM taking into account the reducing power of the residual BSM present in the supernatant (see above). It was assumed that the 22% sialic acid still bound was uniformly distributed between the BSM of the supernatant and that of the sediment as indicated by the positive direct Ehrlich reaction of the sediment.

(c) *Effect of RDE pretreatment of BSM*: 200 mg BSM, dissolved in 20 ml 0.01% $CaCl_2$ solution, were digested for 6 h with 0.25 ml RDE solution ($2 \cdot 10^5$ units) at 37° and pH 7.0. After exhaustive dialysis the residual mixture was freeze-dried and an aliquot treated as under (b). Reducing power, referred to 90 mg freeze-dried material: 4.0 mg (as glucose) corresponding to 6.5 mg sialic acid, *i.e.* about 42% of the total. The low yield of sialic acid after pretreatment of BSM with RDE is in agreement with an experiment in which 1.0 g BSM, dissolved in 100 ml 0.01% $CaCl_2$ solution, was digested for 24 h with 1.0 ml RDE solution ($10 \cdot 10^5$ units) at 37° and pH 7.0. This treatment resulted in the release of 64% of the sialic acid as determined colorimetrically by the direct Ehrlich reaction.

The results would indicate that the reducing group of sialic acid within the BSM molecule is engaged in an acid- and alkali-labile glycosidic linkage. If in BSM, as is the case in UM, the acetylated neuraminic acid is located in the carbohydrate prosthetic group, then its glycosidic partner must be predominantly galactosamine, as is evident from the low non-amino sugar (2.2%) and glucosamine (0.8%) content of BSM[11] and its high galactosamine value (9.2%). Available evidence does not allow one to decide whether the glycoside is of the O- or of the N-type. However, an O-glycosidic linkage of the normal type as in methoxy neuraminic acid[5] may be excluded because of its alkali stability. The viral enzyme and RDE may now be classified as glycosidases; their specificity requirements have yet to be established.

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[1] G. BLIX, *Z. physiol. Chem.*, 240 (1936) 43.
[2] E. KLENK, H. FAILLARD AND H. LEMPFRID, *Z. physiol. Chem.*, 301 (1955) 235.
[3] A. GOTTSCHALK, *Biochem. J.*, 61 (1955) 298.
[4] For literature review see A. GOTTSCHALK, *Yale J. Biol. and Med.*, 28 (1956) 525
[5] A. GOTTSCHALK, *Nature*, 176 (1955) 881.
[6] G. BLIX, E. LINDBERG, L. ODIN AND I. WERNER, *Nature*, 175 (1955) 340.
[7] I. WERNER AND L. ODIN, *Acta Soc. Med. Upsaliensis*, 57 (1952) 230.
[8] A. GOTTSCHALK, *Yale J. Biol. and Med.*, 26 (1954) 352.
[9] A. GOTTSCHALK, *Nature*, 170 (1952) 662.
[10] A. W. VAN DER HAAR, *Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren*, Borntraeger, Berlin, 1920.
[11] A. GOTTSCHALK AND G. L. ADA, *Biochem. J.*, 62 (1956) 681.

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