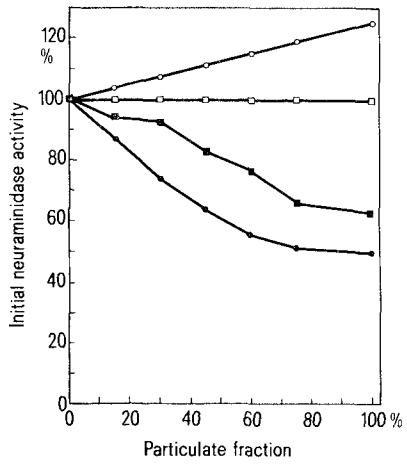

Inhibition of soluble chick-liver neuraminidase by increasing concentrations of active and heat-inactivated particulate neuraminidase. A constant amount of soluble enzyme was present in all incubation mixtures. Ordinate values represent the activity of the mixtures expressed as percent of the activity given by the soluble fraction by itself. Abcissa values indicate percent of active (●) and heat inactivated (■) particulate fraction added to the mixture; at 100%, soluble and particulate fractions are present in the same proportion found in the original fresh tissue. All assays were conducted using neuramin-lactose (2→3′) as the substrate; the data shown corresponds to the average of two separate experiments. Theoretical values, calculated by adding the activities of the soluble and particulate enzymes assayed separately, are included for mixtures containing active (○) and heat-inactivated (□) particulate preparations.

No destruction of free NANA (NANA-aldolase activity), or interference with the thiobarbituric acid assay, by the particulate fraction could be detected.

No marked differences were observed in the pH-versus activity curves of the cytoplasmic extract, soluble and particulate enzymes.

No abnormal behavior was observed upon determination of neuraminidase activity with increasing substrate concentrations. The K_m values for neuramin-lactose (2→3′) were of the same order of magnitude: soluble neuraminidase 1.20×10^{-3} *M*; particulate neuraminidase 2.72×10^{-3} *M* and cytoplasmic extract 1.97×10^{-3} *M*.

Experiments with [^{14}C]-U-neuramin-lactose[9] indicated absence of irreversible binding of the substrate by the particulate fraction and ruled out possible transfer (transglycosylation) of NANA (cleaved from the substrate by neuraminidase action) to endogenous or exogenous acceptors.

Since the cytosolic neuraminidase of chick liver exhibits very low activity towards macromolecules[4], our data suggests the possibility that binding of this enzyme to sialyl groups on the surface of the native, and of the heat-inactivated, particulate fraction may account for the loss of activity observed in these experiments. It is tempting to speculate that a similar phenomenon may also play a role in the regulation of neuraminidase activity in vivo.

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On the Nature of Protein Benzoquinone Complexes[1]

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Summary. Proteins form monosubstituted 1,4-benzoquinone derivatives with an excess of reagent via uniformly coloured charge transfer complexes. Some properties of these compounds are reported.

Several investigations have been made on the reaction of amino acids and proteins with 1,4-benzoquinone (pBQ) purporting the formation of mono- and disubstituted quinones in buffered aqueous solutions of organic solvents[2–6], whereas the nature of these products remains controversial[7,8]. Therefore the present work describes some properties of protein pBQ complexes, analogously prepared to the reaction of amino acids[9].

Materials and methods. Human serum albumin and fibrinogen (Behringwerke), α-casein, gelatin, porcine pepsin (Sigma), bovine insulin (generous gift from Hoechst), and bovine pancreas ribonuclease (Boehringer) were purified from low molecular and protein contaminations by repeated gel filtration on Sephadex G-25 (Pharmacia) and G-75 or G-200 resp., dialyzed exhaustively and lyophilized. Acetylated, alkali denatured, and performic acid oxidized proteins[10] were treated in the same manner. 1.0 ml of protein solution, containing 5–20 mg protein in 10 mmol/l NH_4OH, was mixed with 1.0 ml of 200 mmol/l phosphate pH 6.5 and 1.0 ml of 150 mmol/l pBQ in dimethylsulfoxide (DMSO), leading to a final pH value of 7.9 in 50 mmol/l pBQ, 4.7 mol/l DMSO, and 67 mmol/l phosphate. The reaction was allowed to proceed at 25 °C and terminated by double extraction with diethylether after diverse times. The substituted proteins were purified as mentioned above and compared with the native ones with regard to their solubility (A_{280} and A_{250} nm), spectrum (PMQ II Zeiss), molecular weight (gel filtration on Sephadex G-75 or G-200), and charge (paper electrophoresis 8 h with 7 V/cm in 50 mmol/l phosphate pH 6.5 and barbital pH 8.6 (equipment LKB 3276) and ion ex-

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change chromatography on SE- and DEAE-Sephadex). Titration curves of 10 mg protein in 20 mmol/l sodium hydroxide were obtained between pH 11.6 and 3.5 by down-scale titration with 25 mmol/l sulfuric acid (Radiometer titration assembly). Native and substituted proteins were hydrolyzed by trypsin and pronase (Merck), according to NAKAYA et al.[11], followed by TLC (silica gel G Merck, butanol (−1) 4 – acetic acid 1 – water 1 and phenol 3 – water 1) and disc electrophoresis (7.5% polyacrylamide gel, 7 V/cm, vertical rods).

Results. After a reaction period of 30 min, red products appear. They could not be isolated because of decomposition on evaporation. Their almost uniform spectra resemble those of complexes formed by buffered solutions of amino acids between pH 5.5 and 7.5 with an excess of pBQ in organic solvents[9]. The reaction is first order with respect to both components, suggesting a 1:1 interaction. The spectral bands also agree with the maxima of chloranil amino acid charge transfer complexes[12] and probably en route formed monosubstituted pBQ compounds[8], but they entirely differ from the various colours of isolated 2-(amino acid)-quinones[5,6]. The number of the groups reacting with pBQ within 30 min corresponds to the calculated value only in small stretched molecules (table). In the native state, 4 lysine residues in ribonuclease, 1 in insulin and 16 in albumin are not accessible, human fibrinogen, casein, and gelatin even showing less reactivity.

Reaction of proteins at 25 °C within 30 min, see 'methods'

Original protein or amino acid	max (nm)	Number of reactive sites α-NH_2	ε-NH_2	Indole	ε_{492} calc.	(cm^2/μmol) found
α-Amino acids[a]	490	1	–	–	–	2.75
Nα-Acetyllysine	490	–	1	–	–	1.65
Lysine	490	1	1	–	4.40	4.40
Tryptophan	500	1	–	1	–	3.55
Pepsin C	492	1	4	6	14.15	14.10
Insulin						
oxidized a-chain	490	1	–	–	2.75	2.75
denatured	485	2	1	–	7.15	7.15
native	485	2	1	–	7.15	5.50
Ribonuclease						
oxidized	490	1	10	–	19.25	19.30
native	485	1	10	–	19.25	12.60
Albumin	490	1	56	1	95.95	69.50

[a] Except tryptophan, lysine, proline and cysteine.

Treatment lasting for 2 h leads to dark solutions with absorption maxima between 320 and 350 nm, known from 2,5-disubstituted-pBQ[8], followed by peaks at 230–250 nm after a period of 24 h, indicating the formation of 2-monosubstituted-pBQ[5]. The isolated pBQ protein complexes form hygroscopic brown to red solids, which are throughout more soluble at pH 7.5 and less precipitable by trichloracetic acid than their native analogues. This may be attributed to a higher negative charge by the introduction of quinone residues as indicated by titration curves displaying more titratable groups between pH 8.0 and 10.0, but less above 10.6. This suggests a substitution of side chain amino groups. As compared to the native proteins, the complexes show a 1.3–1.4 times higher anodic mobility in electrophoresis at pH 8.6 and equal velocity at pH 6.5. They are less retarded on SE-Sephadex at pH 7.0, but more on DEAE-Sephadex at pH 8.0, where pBQ-casein and -albumin cannot be eluted by 50 mmol/l triethanolamine. At 25 °C the complexes are stable for at least 10 days against 100 mmol/l sodium hydroxide and sulfuric acid, 5 mol/l urea and up to 50 g/l Tween 20. Pronase exerts identical effects on native and pBQ marked proteins, while peptide maps and disc electrophoretic patterns after digestion with trypsin show reduced cleavage by preceeding treatment with pBQ. Thus blocking of lysine prevents splitting of the b-chain of insulin and the formation of tryptic cores in the hydrolysis of substituted albumin, casein and insulin. As revealed by gel filtration and SDS disc electrophoresis, native proteins and pBQ protein complexes possess the same molecular weights roughly excluding pBQ cross-linking between molecules.

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Synthesis of Bradykinin Potentiating Pentapeptide (BPP_{5a})

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Summary. This synthesis is especially suitable for production of highly-purified bradykinin-potentiating pentapeptide (BPP_{5a}) because of the high yields of the coupling and deprotection reactions, accompanied by minimal side reactions, and the need for only one simple final purification step.

The pentapeptide L-pyroglutamyl-L-lysyl-L-tryptophyl-L-alanyl-L-proline (XIV) has been shown to inhibit the conversion of angiotensin I to angiotensin II and the pulmonary inactivation of bradykinin[2,3]. It was first isolated from Bothrops Jararaca venom[4] and has been characterized and synthesized by a solid-phase procedure[3].

While the solid-phase approach will suffice to provide small quantities of peptide, a classical technique has the advantage of being able to yield gram quantities conveniently and reproducibly. In this paper, a classical and economically-viable, industrial procedure is reported for the synthesis, in high yield, of pure bradykinin-potentiating pentapeptide. In most cases, the intermediate peptides

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