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Masticatory lubrication

The role of carbohydrate in the lubricating property of a salivary glycoprotein–albumin complex

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We report for the first time a masticatory-lubrication assay system to assess the lubricating properties of salivary constituents. The lubricating ability of the proline-rich glycoprotein (PRG) of parotid saliva was enhanced by human serum albumin. The interactive effect of albumin was abolished by chemically deglycosylating the glycoprotein. Fluorescence spectroscopy with a hydrophobic probe verified the existence of a PRG–albumin complex and demonstrated that deglycosylation of the PRG altered the nature of its interaction with albumin.

Acquired enamel pellicle, the organic film that coats the tooth surface, functions in part to provide a lubricating interface between teeth and allow normal functional movements to occur (Rolla, 1983). In order to begin defining the molecular basis of masticatory lubrication, we have initiated studies aimed at elucidating the interactive phenomenon between various salivary constituents. The present study reports that interaction between two pellicle constituents [PRG and albumin (Levine *et al.*, 1985)] facilitates lubrication in an '*in vitro*' friction-testing system. In addition, evidence is presented which demonstrates that the carbohydrate moiety of PRG influences the lubricating ability of the PRG–albumin complex.

Experimental procedures

Preparation of PRG and d-PRG

PRG was purified from the citric acid-stimulated parotid saliva of a healthy 36-year-old male (Levine *et al.*, 1969; Li & Levine, 1980). PRG was deglycosylated by a modification of the method described by Edge *et al.* (1981). A 1 ml portion of anisole (Aldrich Chemical Co.) and 2 ml of trifluoromethanesulphonic acid (Aldrich) were cooled to 0°C, and then 1 ml of this solution was added to 7 mg of PRG in a 2 ml Reactivial (Pierce Chemical Co.). Nitrogen was bubbled through the solution for 30 s, and the reaction was allowed to proceed with stirring at 8°C for 20 h. The reaction mixture was taken up in 4 vol. of diethyl ether precooled in a solid-CO_2/acetone bath. The clear solution was extracted with chilled pyridine/distilled water (1:1, v/v). The aqueous layer was back-extracted twice with cold diethyl ether and dialysed for 2 days at 8°C against 4 litres of 2 mM-pyridine/acetate buffer, pH 5.5. The resulting solution was freeze-dried with water several times. Approx. 4.6 mg of d-PRG was recovered. Amino acid and carbohydrate analyses were carried out by previously described procedures (Reddy *et al.*, 1982). Amino acids and hexosamines were quantified on a Beckman model 120C amino acid analyser. Neutral sugars were quantified as their alditol acetates on a Varian model 3700 gas chromatograph interfaced to an Apple IIe computer through a Gilson model 620 data module. Electrophoresis of native and d-PRG was performed on slabs of SDS/10%-(w/v)-polyacrylamide gel as described by Laemmli (1970). Gels were then analysed on a LKB model 2202 He/Ne-laser scanning densitometer. The amino acid composition of the d-PRG was identical with that of native PRG (Table 1); furthermore, SDS/polyacrylamide-gel electrophoresis showed that both native and d-PRG gave a single band with R_m values of 0.33 and 0.55 respectively (see Fig. 1). d-PRG was found to have no neutral sugars (e.g. fucose, mannose and galactose), but retained six *N*-acetylglycosamine residues, presumably those linked directly to asparagine in the polypeptide

Abbreviations used: PRG, proline-rich glycoprotein; d-PRG, deglycosylated PRG; HSA, human serum albumin; ANS, 1-anilinonaphthalene-8-sulphonate; SDS, sodium dodecyl sulphate.

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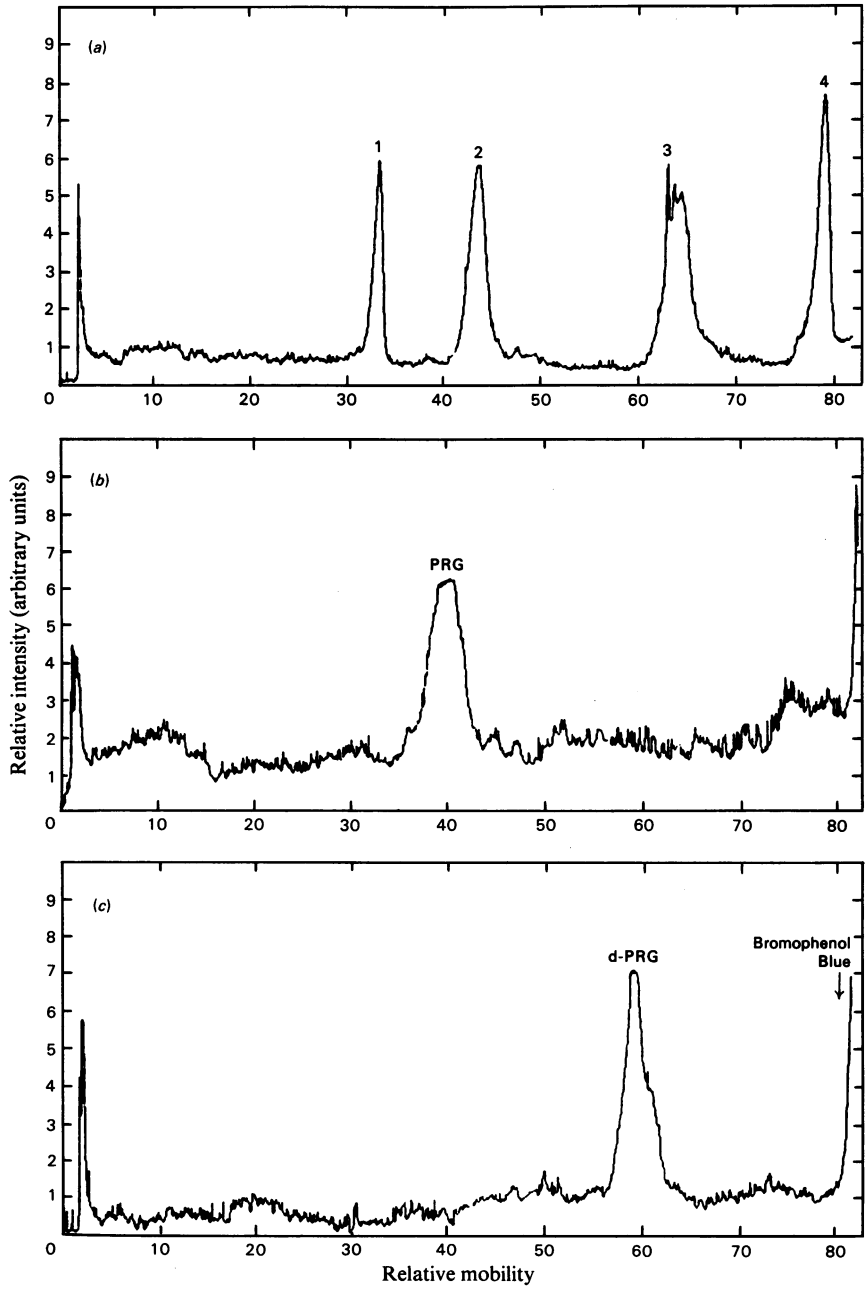

Fig. 1. *Densitometer scan from SDS/10%-polyacrylamide-gel electrophoresis of standard proteins (a), intact PRG (b) and d-PRG (c)*

Approx. 35 μg of PRG and d-PRG were applied to the gel. 1, Phosphorylase *b* (94kDa); 2, bovine serum albumin (67kDa); 3, ovalbumin (43kDa); 4, carbonic anhydrase (30kDa).

backbone (Reddy *et al.*, 1982). These results indicate that the peptide of d-PRG was intact, with only the hexosamines directly attached to the backbone remaining. HSA (essentially fatty-acid-free) [less than 0.5% (w/w) as determined by previously described methods (Tabak *et al.*, 1984)] was obtained from Sigma Chemical Co. and used without further purification.

Measurement of relative lubrication

A modification of a friction-testing device described by Swann *et al.* (1981) was used. The

Table 1. *Amino acid compositions of intact and d-PRG*

Amino acid	Composition (mol/100mol) Intact PRG	Deglycosylated PRG
Proline	32.3	31.2
Glycine	21.9	21.4
Glutamic acid	21.1	20.0
Aspartic acid	5.7	6.0
Arginine	5.5	5.7
Serine	5.3	5.6
Lysine	4.9	5.6
Histidine	1.1	1.1
Valine	0.7	0.5
Alanine	0.6	0.6
Leucine	0.5	1.2
Threonine	0.3	0.1
Isoleucine	0.2	0.7
Methionine	0	0
Half-cystine	0	0
Phenylalanine	0	0
Tyrosine	0	0

incisal incline of a cleaned caries-free human cuspid tooth served as an interface with a rotating glass plate. The rotation of the glass plate was increased incrementally from 8, 12, 16, 24 and 30rev./min after a 1ml portion of test solution had been applied to the glass plate. Transducer output (0–10mV) was recorded on a strip-chart recorder as a function of glass-disc rotation. Glycerol (Fisher Scientific Co.) (0–0.5mV) and salivary buffer [2.1mM-sodium phosphate/36mM-NaCl/0.96mM-$CaCl_2$, pH7.3 (Bennick & Cannon, 1978)] (9–10mV) served as positive and negative controls respectively. An applied load of 50g was used in all experiments, since this weight gave the most consistent difference between positive and negative control solutions. All friction-test experiments were performed at 25°C in a humidified atmosphere as described previously (Swann *et al.*, 1981). Test solutions of HSA, PRG and d-PRG were prepared at a concentration of 2.62nmol of protein/ml in salivary buffer. Mixtures containing 2.62nmol of protein (HSA/PRG and HSA/d-PRG)/ml of salivary buffer were also prepared. All experiments were performed in triplicate. Lubrication activity was also measured on serial dilutions (1:1 to 1:1000) of all test solutions by using salivary buffer as the diluent.

Fluorescence spectroscopy

The fluorescent hydrophobic probe ANS (Aldrich Chemical Co.) was used to evaluate the interactions between PRG, and d-PRG, and HSA. In a recent study, this probe has been utilized to examine the hydrophobic domains of bovine gallbladder mucin (Smith & LaMont, 1984). Free ANS has a fluorescence emission maximum at 540nm; binding of this probe to hydrophobic sites results in a shift in emission spectra to 474nm and a concomitant increase in fluorescence intensity. The ability of d-PRG or PRG to displace the ANS from the ANS–HSA complex was quantified by comparing the fluorescence intensity of the mixtures with the control value after 30min. The fluorescence emission at 474nm of the ANS–HSA complex was defined as the control (Fig. 3 below). Specifically, HSA (4.82nmol of protein) was dissolved in 1ml of salivary buffer containing 1–30nmol of ANS. A 1ml portion was transferred to a 1cm^2 cuvette and complex-formation was allowed to proceed for 30min at 25°C. PRG or d-PRG (4.82nmol of protein equivalents) was then added to the HSA–ANS mixture. After 30min the displacement of ANS by PRG or d-PRG was assessed by comparing emission spectra recorded over the 400–700nm range (Fig. 3 below).

Fluoresence spectra were recorded on a Perkin–Elmer model 650-40 fluorescence spectrophotometer interfaced through a Cyborg model 42A ISAAC analog-to-digital convertor to an Apple IIe computer. All spectra were corrected to Rhodamine B and compensated for solvent and/or background with a Perkin–Elmer model 650-0265 ordinate data processor. Both excitation and emission slits were set at 10mm. The excitation wavelength was set at 365nm.

Results and discussion

The lubrication properties of saliva have traditionally been ascribed to mucin glycoproteins containing *O*-linked carbohydrate units (Tabak *et al.*, 1982). Although salivary mucins do have lubricating activity (Hatton *et al.*, 1985), the present study indicates that PRG, the major *N*-linked glycoprotein of serous parotid saliva, also displays a lubricating function. The relative lubricating abilities of the test solutions containing 2.62nmol of protein are shown in Table 2. HSA, d-PRG and HSA–d-PRG showed similar lubrication profiles (6–8mV transducer output). A small relative increase in lubricating ability was observed with PRG (4–5mV). In contrast, the HSA–PRG complex revealed a substantial increase in relative lubricating ability compared with the other test solutions (1.9–3.1mV). The relative lubricating activities of the diluted test solutions are shown in Fig. 2. The PRG–HSA complex was a consistently better lubricant at all concentrations than PRG alone, d-PRG or the d-PRG–HSA complex. In addition, all solutions demonstrated a decrease in relative lubrication with increasing dilution.

The displacement of the fluorescent probe ANS from hydrophobic domains on HSA was used to

Table 2. *Relative lubricating activity of test solutions*
Values are means ± S.D. for three experiments. The recorder response is invariant over the speeds tested (8–30 rev./min).

Sample	Recorder response (mV)
Glycerol	0.5 ± 0.5
PRG/HSA	2.5 ± 0.6
PRG	4.5 ± 0.5
d-PRG/HSA	6.5 ± 0.5
d-PRG	6.7 ± 0.5
HSA	7.1 ± 0.8
Salivary buffer	9.4 ± 0.3

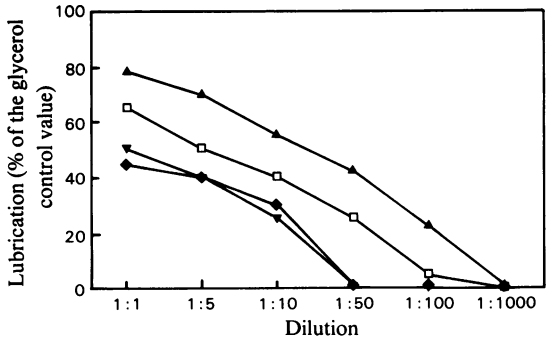

Fig. 2. *Effect of dilution on lubricating activities of test solutions (1.31 μmol/ml of salivary buffer)*
□, PRG; ▲, PRG+HSA; ◆, d-PRG; ▼, d-PRG+HSA.

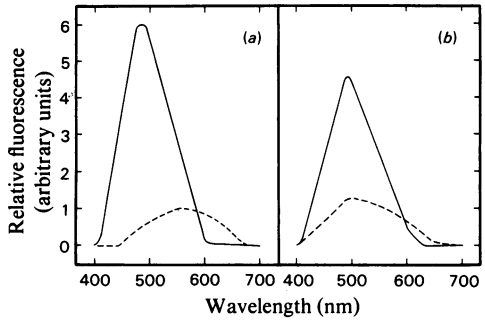

Fig. 3. *Fluorescence-emission spectra (excitation wavelength 365 nm) of (a) ANS (----) and HSA–ANS complex (——) and (b) HSA–ANS complex after displacement of ANS with d-PRG (----) and HSA–ANS complex after displacement of ANS with PRG (——)*

demonstrate PRG–albumin complexing and the role of PRG's carbohydrate units in this interaction (Fig. 3). The addition of PRG to the ANS–HSA complex resulted in a 23% displacement of ANS, whereas d-PRG was able to displace 79% of the ANS. Since the d-PRG was shown to compete more successfully than native PRG for ANS binding sites on HSA, the interaction must be in part predicated upon the presence of intact *N*-linked carbohydrate chains. d-PRG could interact to a greater extent with HSA because the steric and thermodynamic restrictions imposed by the hydrophilic carbohydrate chains are no longer present.

The ability of HSA to enhance the lubricating properties of saliva may be of paramount clinical significance. In individuals with compromised salivary output, the incidence of oral infection, dental caries and periodontal disease is increased (Fox *et al.*, 1985). In such instances, artificial salivas have been employed with moderate success. The use of a biocompatible compound such as HSA as an ingredient in artificial saliva might improve the protective properties of this therapeutic agent.

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