# Suppression of ultrafast supercontinuum generation in a salivary protein

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**Abstract.** The first studies of the propagation of ultrafast ($<45$ fs) pulses of intense infrared light through protein media reveal that supercontinuum (white light) generation is severely suppressed in the presence of the protein $\alpha$-amylase, a potential stress marker in human saliva. The continuum suppression capacity is attributed to the electron scavenging property of the protein. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2731316]

Propagation of ultrashort, intense, infrared light through matter leads to the visually spectacular phenomenon known as supercontinuum (white light) generation, where the spectrum of transmitted light is considerably broader than that of incident light.[1,2] Another manifestation of propagation effects is the spatio-temporal localization of optical energy that results in formation, within the irradiated media, of bright light streaks known as filaments.[3] While the basic physics behind such propagation effects continues to be the subject of intense inquiry, numerous applications have been found, ranging from control of atmospheric lightening to remote sensing and broadband spectroscopy of atmospheric constituents.[3,4] In condensed media, propagation effects present even richer potential, like bulk modification, laser writing, laser-driven microexplosions, modification of refractive index, and microfluidic devices.[5–7] Propagation effects that ensue upon the irradiation of biological material by ultrashort, intense laser light have not been studied and constitute the subject matter of this work. We report the results of studies on propagation of ultrashort ($<45$ fs) light pulses of 820-nm wavelength through biological media. We focus attention on supercontinuum generation in water that contains different concentrations of the protein $\alpha$-amylase. Our results reveal an as-yet unsuspected facet of white light generation: it appears to be highly susceptible to changes in concentration of this particular protein. Our interest in $\alpha$-amylase stems from the importance of this enzyme as a potential marker of stress experienced by humans.

There is increasing evidence that physiological and psychological stress induces changes in $\alpha$-amylase activity in human saliva. A direct relationship between salivary $\alpha$-amylase and stress markers like catecholamines has been shown.[8] This is of importance, as $\alpha$-amylase is endogenous[9] and has little or no chemico-physiological relationship with catecholamines and other stress markers like cortisol; salivary $\alpha$-amylase may turn out to be an *additional*, independent parameter in measurements of stress in humans. Microarray experiments have shown that $\alpha$-amylase production in microbial cultures also enhances with oxidative stress.[10] Attempts have been made to develop sensors based on $\alpha$-amylase activity to quantify stress.[11] We note that even in plant systems, early work revealed that although water-induced stress does not significantly change total protein synthesis, it does enhance $\alpha$-amylase mRNA, indicating that regulation of gene expression might well result from metabolic changes caused by water-induced stress.[12]

In our experiments on $\alpha$-amylase and other proteins, supercontinuum generation was by a Ti-sapphire laser (820-nm wavelength pulses, duration $<45$ fs, 1-kHz repetition rate) with incident power $75\pm5\%$ $\mu$J, corresponding to $600\,P_{cr}$ (critical power for self-focusing in water, $P_{cr}=2.7$ MW). A 30-cm lens loosely focused the incident light onto a 1-cm quartz cuvette containing water and $\alpha$-amylase with typical intensities $\sim1$ TW cm$^{-2}$. Spectral characterization of the transmitted light was by an integrating sphere attached to a fiber-optic-coupled spectrometer (wavelength range 200 to 870 nm).[13] Commercial salivary $\alpha$-amylase was obtained from Sigma-Aldrich (Bangalore, India), and pancreatin was from Sisco Research Laboratory (Mumbai, India). We also made measurements in which the enzyme was from humans; concentrations were then determined by measuring enzyme degradation products kinetically using enzyme-catalyzed reactions. We used the well-proven cleavage of 4,6-ethylidene-$(G_7)$–1,4-nitrophenyl-(G1)-$\propto$, D-maltoheptaoside [ethylidene-protected-substrate (EPS)] by $\propto$-amylase, and subsequently hydrolyzed all the degradation products to p-nitrophenol with $\propto$-glucosidase.[14] The color intensity of p-nitrophenol, directly proportional to $\propto$-amylase activity, was photometrically measured with an autoanalyzer (Hitachi 912). Measurements were made on the solutes used in this study to confirm zero absorption at the incident wavelength centered at 820 nm.

A typical white light spectrum obtained with triply distilled water (no $\alpha$-amylase) has two components [Fig. 1(a)]: 1. one due to self-phase modulation (SPM) that arises from the Kerr nonlinearity and results in broadening that is essentially symmetric about the incident laser wavelength; and 2. an asymmetric component on the blue side of the spectra, which is attributed to the space-time focusing, self steepening,[15] and plasma formation[13,16] that arises from multiphoton-induced (MPI) free electrons. Kandidov et al.[16] have modeled such asymmetric broadening by considering separately the contributions to the refractive index from the Kerr nonlinearity $\Delta n_k$ and the plasma component $\Delta n_p$: $\delta\omega(\tau)=-kz\,\partial[(\Delta n_k)+(\Delta n_p)]/\partial\tau$, where $z$ denotes the propagation axis, $k$ is the wave number, and $\tau$ denotes time. Kolesnik et al.[17] have shown that in water, linear chromatic dispersion plays a major
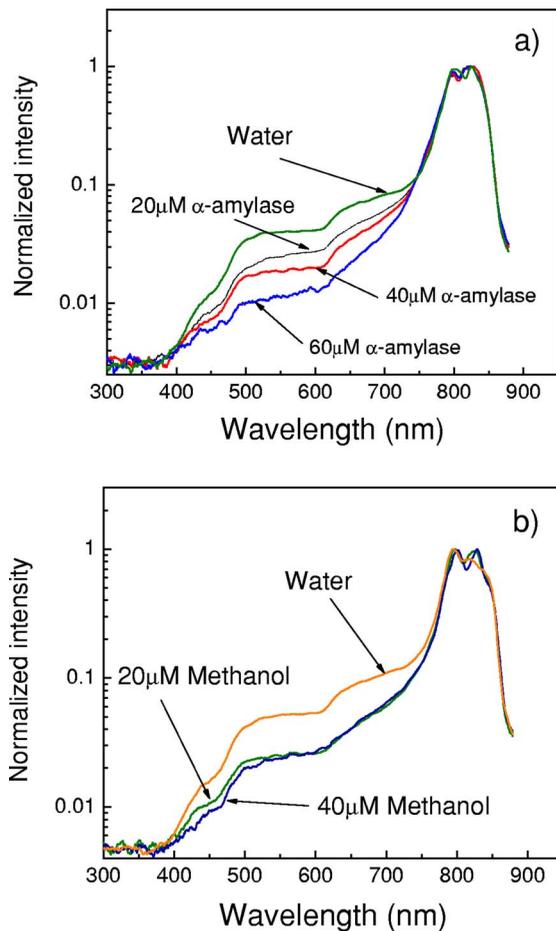
**Fig. 1** (a) White light spectrum of pure $H_2O$ and $H_2O$ + $\alpha$-amylase. Note the suppression of white light as the protein concentration is increased. For 60-$\mu$M concentration, the suppression factors at 500 and 600 nm were 3.4 and 3.2, respectively. The incident 820-nm light had a spectral bandwidth of 30 nm. (b) White light spectrum obtained when methanol, an electron scavenger, is added to water.



**Fig. 2** White light spectrum obtained with pure $H_2O$ and $H_2O$ + immunoglobulin (IG) at an incident laser intensity of ~1 TW cm$^{-2}$. For 40-$\mu$M concentration, the suppression factor was 2.1 at 500 and 600 nm. Similar results were obtained with lysine and arginine.

role in suppression of supercontinuum, in addition to MPI and plasma defocusing.

In addition to asymmetric broadening, Fig. 1(a) also shows a marked dip at 630 nm that is due to an inverse Raman effect,[18] wherein upon simultaneous irradiation of a sample by intense monochromatic light of frequency $\nu_0$ and an intense continuum, $H_2O$ is stimulated to radiate at $\nu_0$ and, at the same time, absorb at $(\nu_0 + \nu_m)$ and $(\nu_0 - \nu_m)$, where $h\nu_m$ is the Raman shift. The relatively sharp and distinct dip superimposed on the continuum is due to absorption on the higher frequency side.

Changes in white light spectra occur on the addition of $\alpha$-amylase [Fig. 1(a)]. As the enzyme concentration changes (20 to 60 $\mu$M), the intensity of spectral broadening markedly decreases at constant incident laser power. This is also reflected in extinction of the inverse Raman dip. With 60-$\mu$M $\alpha$-amylase, the remnant broadening that is observed is almost symmetrical. Plasma effects, one of the causes of spectral asymmetry, are almost totally extinguished, most likely as a result of $\alpha$-amylase acting as a strong electron scavenger. This action involves dissociative attachment (DA) of MPI-induced electrons, a large cross section ($\sim 10^{14}$ cm$^{-2}$) process for low-
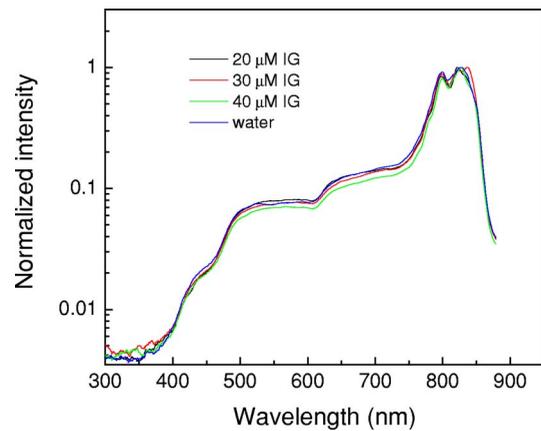
energy ($<5$ eV) electrons. In terms of a generic molecule ABC, DA may be represented as e+ABC$\rightarrow$[ABC]$^-\rightarrow$A$^-$+BC, where the intermediate stage is a short-lived negative ion whose dissociation products are a free radical, BC, and a negative ion A$^-$. The role of free radicals, like atomic oxygen, in physiological functions and malfunctions is established and has been cogently reviewed recently.[19] In case of water plus $\alpha$-amylase, as soon as free electrons are generated by MPI, they are rapidly and efficiently scavenged by $\alpha$-amylase via DA. The negative contribution to $\partial n$ is diminished by quenching of free electrons; a symmetrically broadened spectrum results. The process may also be envisioned in the following terms: on entering the protein sample, the leading part of the incident 820-nm pulse experiences refractive index change due to the Kerr effect that increases with time; frequencies arise that are lower than the incident frequency (the Stokes region). The situation reverses in the trailing part of the pulse: higher frequencies are generated (the anti-Stokes region). The net result is broadening that is symmetric around 820 nm. If $\alpha$-amylase was not scavenging electrons, the MPI-generated electrons would also contribute to spectral broadening; $\delta\omega$ would become positive because of the negative contribution to $\partial n/\partial\tau$. Broadening would now be asymmetric, with a higher propensity for generation of frequencies higher than 820 nm (anti-Stokes broadening).

We checked the veracity of our model by measuring white light spectra obtained by adding methanol, a well-known electron scavenger, to water. The results [Fig. 1(b)] were identical to those obtained using $\alpha$-amylase.

Do other proteins display the same properties as $\alpha$-amylase? We have carried out measurements of white light spectra using water and immunoglobulin (Fig. 2), lysine, and arginine. All spectra were found to be identical to that in pure water, including the inverse Raman feature. The spectral modification depicted in Fig. 1(a) appears to be a special feature of $\alpha$-amylase and is of relevance to optical sensing of a stress marker. We also made white light measurements on samples in which $\alpha$-amylase was extracted from human saliva and the pancreas. Unstimulated human saliva contains electrolytes, immunoglobulins, proteins, enzymes, mucins, and ni-
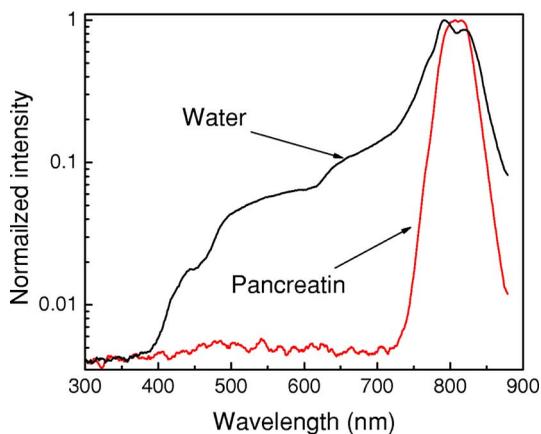
**Fig. 3** White light spectrum obtained with pure $H_2O$ and $H_2O$ + pancreatin. The white light suppression factors at 500 and 600 nm were 8.3 and 13.3, respectively.

trogenous products.[20] Of the 30 odd enzymes in saliva, amylase is the best known; it catalyzes breakdown of starch.[21] S-amylase activities are known to elevate in diverse stress situations.[22] The pancreas also secretes amylase in the highest concentration and largest total amount of any human organ.[22] Noting the relationship between stress and salivary $\alpha$-amylase, we made measurements using human-salivary and pancreatic $\alpha$-amylase samples. Figure 3 shows typical spectra measured using pancreatin (another source of $\alpha$-amylase); similar observations were made using human salivary $\alpha$-amylase. Very dramatic morphological change is seen in the spectra, in accord with data shown in Fig. 1(a).

In summary, we investigated propagation of ultrashort, intense, infrared light pulses through biologically interesting media. Supercontinuum generation that occurs in water (along with an accompanying inverse Raman spectral feature) is severely suppressed in the presence of micromolar concentrations of $\alpha$-amylase. In our study, we did not find such suppression with other related proteins. It is of interest to note that pioneering work on ultrafast supercontinuum generation in water carried out by Alfano et al. (see Ref. 1 and references therein) revealed a measure of enhancement upon addition of inorganic dopants like $Zn^{2+}$ and $K^+$. Studies of supercontinuum generation in protein media might offer new insights into propagation effects in biological media, and provide different vistas for new diagnostics.

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