Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 320 (2004) 213–217
BBRC
www.elsevier.com/locate/ybbrc

Albumin-bound polyacrolein: implications for Alzheimer's disease

Norbert W. Seidler* and George S. Yeargans

Department of Biochemistry, Kansas City University of Medicine and Biosciences, 1750 Independence Avenue, Kansas City, MO 64106, USA

Received 21 May 2004
Available online 9 June 2004

Abstract

Acrolein-modified proteins are markers of disorders such as Alzheimer's disease (AD). Acrolein ($H_2C{=}HC{-}CH{=}O$), which can be produced by the oxidative properties of amyloid-β (Aβ) peptide, localizes to areas immediately surrounding early Aβ aggregates. The focal production of acrolein would consequently yield localized high concentrations that may be susceptible to polymerization via basic latex polymer chemistry. Using albumin as our model we examined whether simple in vitro conditions may bring about higher order aggregates composed of polyacrolein. We observed that thin plastic-like fragments were formed following incubation of albumin in acrolein solutions from 5 to 500 mM in sodium phosphate buffers (pH 7.4). The layered plastic film stained for carbonyls and for amyloid (cross-β structures) suggesting a polyacrolein–albumin colloidal mixture. Large structures (up to 2700 μm^2) readily form under simple conditions. These observations suggest that polyacrolein latexes may potentially exist in biological tissues contributing to the pathogenesis of diseases such as AD.

Keywords: Acrolein; Albumin; Alzheimer's disease; Light microscopy; Polyacrolein

Alzheimer's disease (AD) brain has high levels of acrolein [1], which is a toxic α, β-unsaturated aldehyde product of lipid peroxidation that damages proteins. Protein-bearing carbonyls correlate with the pathogenesis of AD [2]. Acrolein is a marker of oxidative stress and localizes to neurofibrillary tangles and dystrophic neurites surrounding amyloid-β (Aβ) aggregates [3]. 4-Hydroxy-*trans*-2-nonenal, another α, β-unsaturated aldehyde product of lipid peroxidation, also localizes to the major lesions in AD [4,5] as well as to serum amyloid fibrils [6] derived from immune reactive proteins. Aβ peptide (1–42), an AD plaque component, promotes lipid peroxidation and α, β-unsaturated aldehyde production [7]. These previous studies suggest a relationship between amyloidogenesis and lipid peroxidation. In this study we propose that acrolein may incorporate in plaques by a currently unrecognized mechanism.

Among the α, β-unsaturated aldehydes, acrolein is the most electrophilic reacting with sulfhydryls, imidazoles, and primary amines [8]. Acrolein inhibits diverse enzymes [9–11]. However, the role of acrolein in promoting protein aggregation, which is the hallmark of AD, is largely unknown. Many neurodegenerative disorders are due to protein aggregation [12]. These misfolding diseases include a large group of diverse proteins and exhibit protein deposits that are fibrillar or amorphous and that contain cross-β structures typically found in amyloid fibrils.

Protein glycation (or sugar-mediated protein modification), which is implicated in AD [13], promotes aggregation and cross-β structures in albumin [14]. We similarly chose albumin in the current study to look at acrolein-dependent amyloidogenesis. Our novel findings are that acrolein-based latexes can form without surfactant or radiation, which are the typically used catalysts for polymerization. We discuss the biological significance of protein-bound polyacrolein.

Materials and methods

Materials. Human serum albumin (HSA), acrolein, Congo red, and 2,4-dinitrophenylhydrazine (DNPH) were purchased from Sigma Chemical (St. Louis, MO). Bovine serum albumin (BSA), Cohn

* Corresponding author. Fax: 1-816-283-2357.
E-mail address: nseidler@kcumb.edu (N.W. Seidler).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.05.154

fraction-grade C, was obtained from Calbiochem (EMD Biosciences, La Jolla, CA). All other chemicals were of reagent grade.

Incubation conditions. HSA samples were prepared in a 50 mM sodium phosphate buffer (pH 7.4) containing various concentrations of acrolein (0.005–1.0 M) and placed directly onto 96-well Costar microplates (Corning, Corning, NY) for microscopic and turbidometric analysis. Samples were kept at room temperature and incubation time was varied.

Light microscopy. Samples, which included appropriate controls, were kept in microplates and directly observed using a Nikon Eclipse TE2000-U inverted microscope with phase contrast at various magnifications (60–300×). Images were obtained and examined using AnalySIS Computer Program (Soft Imaging System, Lakewood, CO).

Turbidity measurements. Protein aggregation of HSA samples was assessed turbidometrically by measuring light scattering, which was performed using a BioRad microplate reader-model 550 (Hercules, CA) with a 595 nm filter. Light transmittance was determined at various acrolein concentrations and compared versus control.

Preparation and analysis of BSA–acrolein aggregates. BSA samples (20 mg/mL) were incubated in a 50 mM sodium phosphate buffer (pH 7.4) containing acrolein (1.0 M), placed in cell culture flasks (Corning, Corning, NY), and kept at room temperature for 1 h prior to analysis. Samples were centrifuged (1315*g*) in an analytical centrifuge for 5 min. The sediment was washed twice with a 50 mM sodium phosphate buffer (pH 7.4) and the final resuspension was in 50 mM sodium phosphate (pH 7.4). Protein concentration was measured spectrophotometrically by determining the 260/280 nm absorbances of an aliquot dissolved in 1 N NaOH using an Ultrospec 4000 UV/Vis spectrophotometer (Amersham Biosciences, Piscataway, NJ). Carbonyl content was determined by DNPH labeling followed by microscopic analysis. Presence of amyloid-like conformation was assessed by Congo red staining. An aliquot of the resuspension (50 μL) was added to 200 μL Congo red solution, which was prepared by prior mixing of 300 μM Congo red in 90% PBS/10% ethanol and passing through a 0.2 μm filter. PBS was a 10 mM sodium phosphate buffer (pH 7.4) containing 2.7 mM KCl and 137 mM NaCl. Samples were incubated for 15 min at room temperature and then centrifuged (3000*g*) for 10 min, washed once with PBS, and resuspended in a 1 mM sodium phosphate buffer (pH 7.0) containing 40% ethanol. The resuspension was examined spectrophotometrically and microscopically.

Results and discussion

Acrolein modifies intracellular enzymes [10,15] as well as serum proteins such as albumin [16,17], forming adducts and crosslinked products. We examined the effects of acrolein-induced albumin modification on aggregation, since amyloid results from glycation (1 M glucose 6-phosphate, 23 weeks, 37 °C) of albumin [14] and albumin gelation occurs upon heating [18]. These studies [14,18] present albumin-derived fibrillar aggregates that measure up to 300 nm only seen using electron microscopy. We report that acrolein promotes the formation of amorphous planar polyacrolein–albumin aggregates with dimensions exceeding 100 μm that is visible under light microscopy.

We observed that acrolein (5–100 mM) increased the light scattering of albumin mixtures in a concentration-dependent manner (Fig. 1). These data suggest that under these conditions maximum aggregate formation occurred at approximately 50 mM acrolein. We then

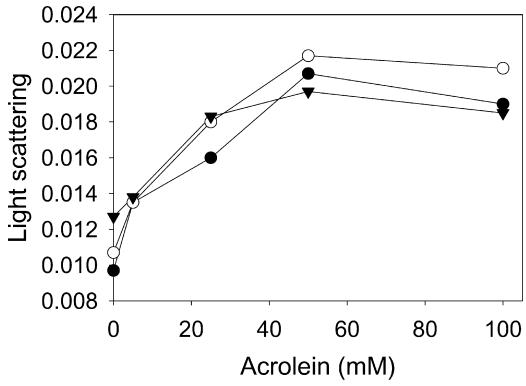

Fig. 1. Effects of acrolein on light scattering. HSA samples were incubated at room temperature in various concentrations of acrolein and absorbances (595 nm) were obtained at 1 day (closed circles), 3 days (open circles), and 7 days (triangles). Data represent multiple readings from a representative experiment.

examined the mixtures under light microscopy and observed structures that resembled plastic-like fragments (Fig. 2). Even at 5 mM acrolein (24 °C, 10 mg albumin/mL) the plastic-like fragments were visible after 3 days (data not shown). Additionally, when albumin solutions (10 mg/mL) were pipetted into a 500 mM acrolein solution at 24 °C, similar structures rapidly formed (Fig. 3). The size and structure of these thin flat transparent pieces of plastic-like material appeared concentration dependent. As the concentration of acrolein increased from 5 to 100 mM, the size of the fragments increased and the degree of folding and compaction of the layers increased.

Upon harvesting the aggregates by centrifugation, we observed that the sedimented material contained protein

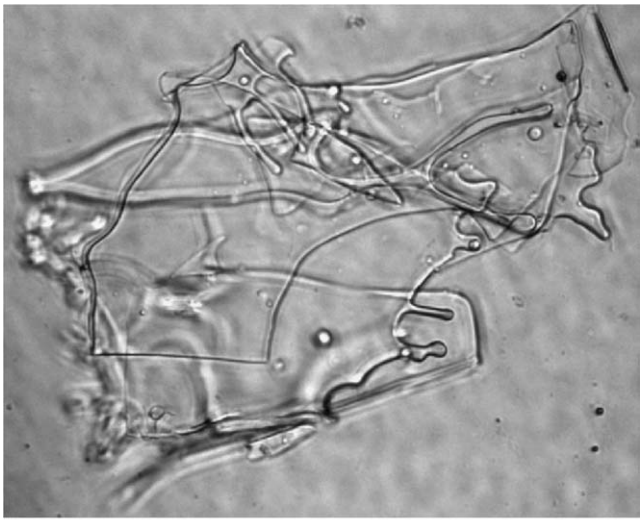

Fig. 2. Formation of plastic-like fragments. HSA (10 mg albumin/mL) was incubated in a 100 mM sodium phosphate buffer (pH 7.4) containing 25 mM acrolein (24 h at 24 °C) prior to phase contrast light microscopy (60×; fragment approx. 2700 μm^2).

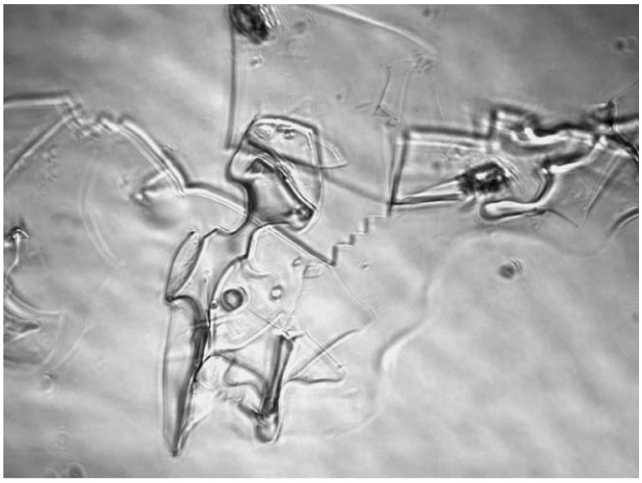

Fig. 3. Albumin catalyzes the formation of plastic-like fragments. HSA solutions (10 mg/mL) were pipetted into a 500 mM acrolein solution in 96-well microplates at room temperature and immediately examined under phase contrast light microscopy (150×; fragment approx. 1800 μm^2).

(starting mixture, 50 mg protein/mL in 12 mL; final resuspension, 12.8 mg protein/mL in 150 μL), suggesting that the fragments of plastic film were protein-containing colloids. These fragments were also stained with DNPH (Fig. 4), a carbonyl reagent, demonstrating the presence of acrolein. Accessible carbonyls persist following the polymerization of acrolein monomers across the alkene bonds in creating polyacrolein (Fig. 5). Some carbonyls would be available for Schiff base reaction with albumin, leaving others reactive to DNPH. Alternately, there may be incorporation of acrolein into albumin via Michael additions leaving accessible carbonyls as protein adducts. We also observed that the plastic-like fragments were stained with Congo red (Fig. 6), suggesting that these aggregates contain albumin that may exist in cross-β structures similar to amyloid.

Interestingly, the drug targeting literature describes the use of microencapsulation techniques and the manufacture of microparticles, which may be of relevance to our findings. In the presence of an initiating catalyst acrolein forms polyacrolein first as a thin film that upon processing (i.e., repeated centrifugations) develops into bead-like structures [19]. These latex structures form by emulsion polymerization through acrolein's alkene group and via carbonyl addition forming complicated crosslinked two-dimensional structures. The resulting polyacrolein microspheres maintain their free aldehyde groups, which may bind drugs or proteins [20].

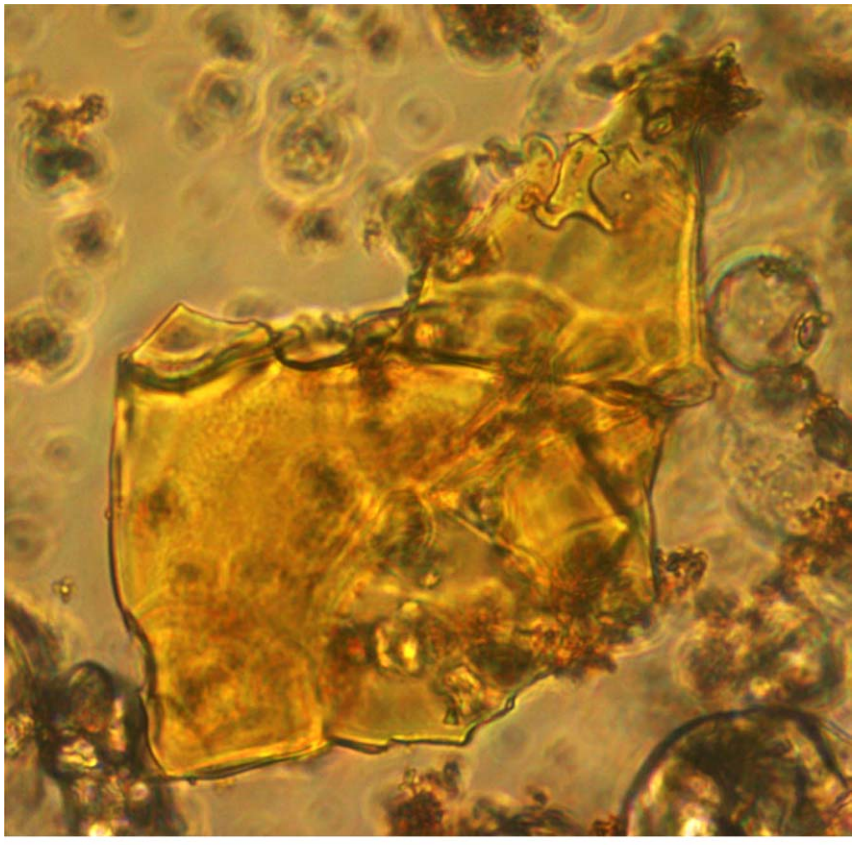

Fig. 4. Fragments stained with DNPH. Following removal of unpolymerized and unbound acrolein, pelleted fragments were stained with the carbonyl reagent DNPH (0.1% in 1 N HCl) and examined under phase contrast light microscopy (300×; fragment approx. 200 μm^2).

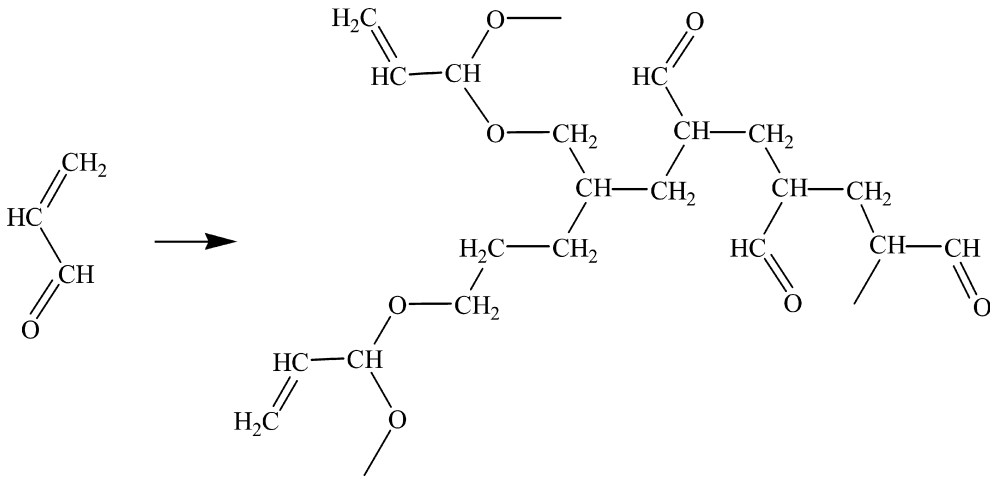

Fig. 5. Proposed polyacrolein structure. Latex structures form by polymerization through acrolein's alkene group and via carbonyl addition forming complicated crosslinked two-dimensional structures.

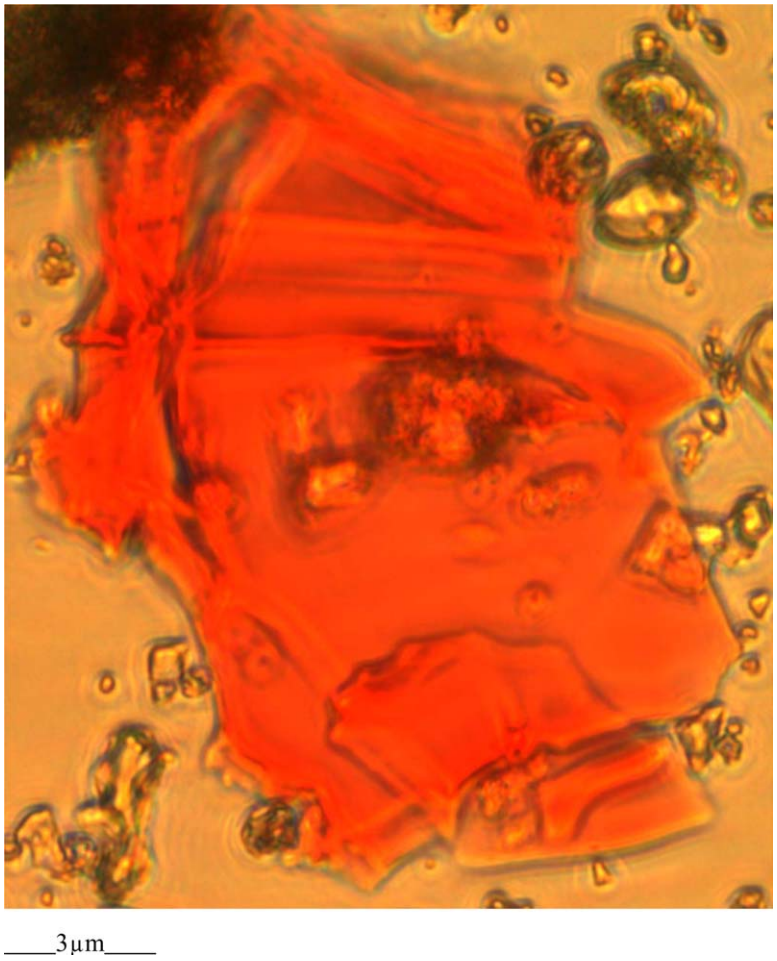

Fig. 6. Fragments stained with Congo red. Following removal of unpolymerized and unbound acrolein, pelleted fragments were stained with the Congo red as described in Materials and methods and examined under phase contrast light microscopy (300×; fragment approx. 190 μm^2).

This study presents compelling data that suggest that polyacrolein may form in the absence of emulsion polymerization techniques that require surfactant and free radicals. We hypothesize that polyacrolein–HSA may form under biological conditions. The presence of polyacrolein–HSA may evoke antigenicity and other cytotoxic processes.

In considering the relevance to AD, in which there is a localized production of Aβ peptide and acrolein, we propose that acrolein may incorporate in plaques via

Schiff base attachment of Aβ onto polyacrolein films. Since Aβ promotes lipid peroxidation and acrolein formation [7], it is reasonable to suggest that the conditions in the diseased nervous system may be suitable for polyacrolein production and incorporation. The appearance of polyacrolein may contribute to the formation and accumulation of the mature plaque.

The composition of the plastic-like material needs further characterization and its presence in vivo requires validation. Nevertheless, we present novel findings suggesting that protein–latex aggregates may form and persist in biological tissues.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Ankur Mehta. The study was supported in part be a grant from the Division of Research at the Kansas City University of Medicine and Biosciences.

References

[1] M.A. Lovell, C. Xie, W.R. Markesbery, Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures, Neurobiol. Aging 22 (2001) 187–194.

[2] C.D. Smith, J.M. Carney, P.E. Starke-Reed, C.N. Oliver, E.R. Stadtman, R.A. Floyd, W.R. Markesbery, Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease, Proc. Natl. Acad. Sci. USA 88 (1991) 10540–10543.

[3] N.Y. Calingasan, K. Uchida, G.E. Gibson, Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease, J. Neurochem. 72 (1999) 751–756.

[4] L.M. Sayre, D.A. Zelasko, P.L. Harris, G. Perry, R.G. Salomon, M.A. Smith, 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease, J. Neurochem. 68 (1997) 2092–2097.

[5] Y. Ando, T. Brannstrom, K. Uchida, N. Nyhlin, B. Nasman, O. Suhr, T. Yamashita, T. Olsson, M. El Salhy, M. Uchino, M. Ando, Histochemical detection of 4-hydroxynonenal protein in Alzheimer amyloid, J. Neurol. Sci. 156 (1998) 172–176.

[6] G. Kamalvand, Z. Ali-Khan, Immunolocalization of lipid peroxidation/advanced glycation end products in amyloid A amyloidosis, Free Radic. Biol. Med. 36 (2004) 657–664.

[7] D.A. Butterfield, A. Castegna, C.M. Lauderback, J. Drake, Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death, Neurobiol. Aging 23 (2002) 655–664.

[8] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1991) 81–128.

[9] C.B. Pocernich, D.A. Butterfield, Acrolein inhibits NADH-linked mitochondrial enzyme activity: implications for Alzheimer's disease, Neurotox. Res. 5 (2003) 515–520.

[10] J.L. Southwell, G.S. Yeargans, C. Kowalewski, N.W. Seidler, Acrolein modifies and inhibits cytosolic aspartate aminotransferase, J. Enzyme Inhib. Med. Chem. 17 (2002) 19–23.

[11] A. Castegna, C.M. Lauderback, H. Mohmmad-Abdul, D.A. Butterfield, Modulation of phospholipid asymmetry in synaptosomal membranes by the lipid peroxidation products, 4-hydroxynonenal and acrolein: implications for Alzheimer's disease, Brain Res. 1004 (2004) 193–197.

[12] M.D. Kaytor, S.T. Warren, Aberrant protein deposition and neurological disease, J. Biol. Chem. 274 (1999) 37507–37510.

[13] X. Girones, A. Guimera, C.Z. Cruz-Sanchez, A. Ortega, N. Sasaki, Z. Makita, J.V. Lafuente, R. Kalaria, F.F. Cruz-Sanchez, *N*(epsilon)-Carboxymethyllysine in brain aging, diabetes mellitus, and Alzheimer's disease, Free Radic. Biol. Med. 36 (2004) 1241–1247.

[14] B. Bouma, L.M. Kroon-Batenburg, Y.P. Wu, B. Brunjes, G. Posthuma, O. Kranenburg, P.G. de Groot, E.E. Voest, M.F. Gebbink, Glycation induces formation of amyloid cross-beta structure in albumin, J. Biol. Chem. 278 (2003) 41810–41819.

[15] Y. Pocker, N. Janjic, Differential modification of specificity in carbonic anhydrase catalysis, J. Biol. Chem. 263 (1988) 6169–6176.

[16] K. Uchida, M. Kanematsu, K. Sakai, T. Matsuda, N. Hattori, Y. Mizuno, D. Suzuki, T. Miyata, N. Noguchi, E. Niki, T. Osawa, Protein-bound acrolein: potential markers for oxidative stress, Proc. Natl. Acad. Sci. USA 95 (1998) 4882–4887.

[17] J.C. Gan, A. Oandasan, G.A. Ansari, In vitro covalent modification of serum albumin by acrolein, Chemosphere 23 (1991) 939–947.

[18] C. Veerman, L.M. Sagis, J. Heck, E. van der Linden, Mesostructure of fibrillar bovine serum albumin gels, Int. J. Biol. Macromol. 31 (2003) 139–146.

[19] M. Chang, G. Richards, A. Rembaum, Polyacrolein microspheres: preparation and characteristics, Methods Enzymol. 112 (1985) 150–164.

[20] S. Margel, Polyacrolein microspheres, Methods Enzymol. 112 (1985) 164–175.