COMPOSITIONS AND METHODS FOR OBTAINING NUCLEIC ACIDS FROM SPUTUM

Background of the Invention

The present invention relates to compositions and methods for preserving nucleic acids at room temperature for extended periods of time and for simplifying the isolation of nucleic acids.

DNA can be extracted from virtually every type of cell in the human body, with the exception of red blood cells. The usual source of bodily samples for extraction of DNA is venous blood, since the number of nucleated white blood cells (principally neutrophils and lymphocytes) is relatively high and quite consistent: the normal range is about 5 to 10 million white blood cells per milliliter of blood. The DNA content of human cells is about 6 micrograms per million cells, so 1 milliliter can theoretically yield from 30 to 60 micrograms of DNA. However, there are about 5 billion red blood cells per milliliter of blood, which, since they contain no DNA, must be removed to obtain pure DNA. Furthermore, the use of blood as a source of DNA has many other disadvantages. Collection of blood is not a trivial procedure. Taking of venous blood requires trained personnel. It is an invasive procedure, which frequently causes some distress and pain to the donor. Precautions are needed to minimize exposure of personnel to blood-borne pathogens. Once collected, the blood sample must be either frozen or quickly transported to a laboratory for extraction of DNA. For these reasons, venous blood is not the ideal source of DNA. A simpler procedure for obtaining blood is to collect a few drops after a finger prick and blotting it onto a piece of filter paper. Less training of personnel is required. Once dried, the DNA is quite stable. The amount of DNA recovered is small but sufficient for many forensic

can be collected by individuals with less training than is required in the collection of blood. Once collected, the time that useable DNA can be recovered can be extended by either drying the swab or wiping onto filter paper and drying it. However, as the inside of the mouth is not a sterile source (as compared to blood) and microbes can degrade the quality of the DNA after a period of time. The number of cells recovered by this procedure is not large and typically less than 1-2 micrograms of DNA can be expected in the entire sample.

Saliva is a fairly clear, colorless fluid secreted principally by the major salivary glands (parotid, submandibular, and sublingual). Its function is to lubricate and cleanse the oral cavity, as well as to initiate the process of digestion. The parotid gland primarily secretes serous (watery) saliva, while the other glands secrete a mixture of serous and mucinous (sticky) saliva. Components of saliva include albumin, globulin, mucins, and digestive enzymes. It has long been known that cellular DNA is present in saliva and that this DNA is suitable for forensic purposes. Forensic use is typically limited to victim or suspect identification, using the tiny amounts of DNA from saliva that may recovered at a crime scene or from the back of a postage stamp. The notion that saliva may be a reliable source of genomic DNA and a rival to venous blood samples for this purpose has been investigated more recently in a scientific publication (van Schie, et al., *J. Immunol. Methods* 208:91-101, 1997). The authors used freshly collected or frozen saliva samples and purified the DNA by a fairly complex extraction procedure. Estimates of the quantity of DNA recovered were based upon light absorption at 260 nm, a procedure known to be an unreliable method since other common biological macromolecules, such as RNA, have essentially the same ultraviolet light absorption spectrum. Nevertheless, these authors showed that quality genomic DNA was indeed present by gel electrophoretic analysis and

was reported, the method used to measure DNA was not. These authors provided 3 examples where saliva dried on filter paper yielded DNA suitable for analysis.

With the increasing use of DNA-based analysis in forensics, law enforcement, military, human medicine, veterinary medicine, and research, there is a need for a product that would allow saliva to become a standard reliable source of DNA from an individual (to replace blood, the current standard). In forensic, military and mass disaster situations, for example, DNA samples are now routinely taken from living persons thought to be relatives of unidentified victims of accident or foul play, to aid in identification of the dead. Military personnel or other individuals who expect to encounter hazardous situations where their lives may be at risk may wish to store DNA samples prior to exposing themselves to these hazards. In the law enforcement area, convicted felons in both Canada and the United States are now required to provide DNA samples. DNA-based tests are expected to increase in medicine, such as testing for cystic fibrosis, cytochrome P450 isotypes, polymorphisms affecting susceptibility to infectious and autoimmune diseases, HLA typing, paternity issues, to name but a few. In clinical studies, an example would be to screen populations for colon cancer-predisposing genes or family members of a breast cancer victim for breast cancer predisposing genes. In all of these cases, there are significant advantages to providing a saliva sample rather than providing a blood sample as a source of DNA. All donors would prefer donating saliva rather than blood because of the discomfort, pain, or apprehension associated with phlebotomy or pin-pricks. Saliva has a further advantage of not requiring specialized personnel thereby reducing cost where mass sample collection is being carried out. The risk of blood-borne infection is likewise decreased.

In addition to the problem of developing a standard collection and

Enzymology 216:154-160, 1993, but this work was not extended to the recovery of nucleic acids from mucin-containing bodily fluids.

Multimeric proteins called mucins are high molecular weight glycosylated proteins that form a major part of a protective biofilm on the surface of epithelial cells, where they can provide a barrier to particulate matter and bind microorganisms. These glycoproteins contribute greatly to the viscoelastic nature of saliva. The major high-molecular-weight mucin in salivary secretions is MUC5B, one of four gel-forming mucins that exist as multimeric proteins with molecular weights greater than 20-40 million daltons. MUC5B is a large oligomeric mucin composed of disulphide-linked subunits.

It is known that reagents that reduce disulfides also reduce the viscosity of mucin, such as that found in sputum or saliva. Reducing agents, in particular sulfur-containing chemicals such as β-mercaptoethanol and dithiothreitol, are widely used in biochemistry. However, many biochemically relevant reducing agents are capable of reacting in solution with dissolved oxygen. This is known are autooxidation (also called autoxidation or auto-oxidation), where 1-electron reduction intermediates of oxygen are formed, *viz.*, superoxide ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). In addition, transitional metal cations function as catalysts and $O_2^{-\cdot}$ has been demonstrated to be an intermediate. Unfortunately, reducing agents and reducing compositions of the prior art have a relatively short shelf life, especially in basic solutions, and stock solutions that contain reducing agents cannot be prepared and stored under ambient conditions for an extended period time, usually not more than a day or two.

Therefore, in addition to a need for a means to collect sputum or saliva, and subsequently preserving the nucleic acids contained therein by contacting them with a stabilizing composition, there is a need for the inclusion of a stable reducing

Summary of the Invention

The present inventor has developed a composition, which, when mixed with a mucin-containing bodily fluid, preserves the nucleic acids at room temperature under ambient conditions for extended periods of time. There is no requirement for freezing of the samples before nucleic acid recovery and purification. The properties of this composition are that it (a) chemically stabilizes nucleic acids, (b) inhibits nucleases that may be present in the saliva, and (c) is compatible with proteolytic enzymes and other reagents used to purify/amplify oligo- or polynucleotides. A fourth and novel property of this composition is that it contains an agent that rapidly reduces the viscous properties of mucin, greatly facilitating the extraction of nucleic acids contained within.

Accordingly, a first aspect of the invention features a composition for preserving nucleic acids that includes a chelating agent, and a denaturing agent, where the pH of the composition is greater than 5.0. In one embodiment, the composition is an aqueous solution.

In another embodiment, the composition also includes a reducing agent. For example, it can include one or more of the following: ascorbic acid, dithionite, erythiorbate, N-acetylcysteine, cysteine, glutathione, dithiothreitol, 2-mercaptoethanol, dierythritol, a resin-supported thiol, a resin-supported phosphine, vitamin E, and trolox, or salts thereof. Desirably, the reducing agent is ascorbic acid, erythiorbate, N-acetylcysteine, dithiothreitol, or 2-mercaptoethanol, and most desirably, the reducing agent is ascorbic acid. In another embodiment, the composition does not contain ascorbic acid. In yet another embodiment, the concentration of the reducing agent in the composition is greater than or equal to 50 millimolar.

Antioxidant free-radical scavengers are also desirable reducing agents for

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